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HYDROGEN-ION DETERMINATION IN FLOUR AND BAKERY PRODUCTS

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Introduction

In connection with the work of these laboratories on the effects of the use of dairy products in breads and cakes, it is necessary to make a large number of determinations of hydrogen-ion concentration. The use for this purpose of a suspension made by allowing the sample to stand in contact with five or ten times its weight of water for 30 minutes at 25°C. with frequent shaking seems to be the generally accepted procedure for flours, and has been used for baked products with the additional feature of separating the extract from the solid residue by decantation. The Bailey (1920) type of hydrogen electrode has been most widely used in this country in cereal work. In several laboratories attempts are being made to use the quinhydrone electrode for this purpose. Halton and Fisher (1928) have used the quinhydrone electrode on flour suspensions, obtaining results that agreed with determinations made with the hydrogen electrode.

The capillary type of quinhydrone electrode developed by Cullen and Biilmann (1925) has been used successfully in these laboratories by Watson (1927) in work on cheese whey. Watson also employed a blade type of gold electrode thrust into mixtures of solid cheese and quinhydrone. As both the capillary and blade quinhydrone electrodes are simpler and quicker than the Bailey hydrogen electrode, and as the blade type does not require the preparation of an extract, thus insuring that the H-ion concentration is not changed previous to measurement, a comparison of results obtained by these three procedures was carried out for a considerable number of baked products and two flour samples.

Methods

For the determinations by the Bailey hydrogen electrode and the capillary quinhydrone electrode, an extract was prepared by suspending 20 grams of crumb in 100 cc. of distilled water, placing the suspension in a water bath held at 25°C. and shaking at frequent intervals for 30 minutes. The suspension was then allowed to settle and the cloudy supernatant liquid poured off and used immediately for the determinations. Three Bailey hydrogen electrodes were plated with palladium black, washed, and saturated with hydrogen. A portion of the extract was introduced into each and hydrogen bubbled in to fill the closed end nearly to the electrode and to force the liquid to fill the open end completely. A glass stopper was then inserted and the electrode vessel was shaken for 2 minutes. Readings of potential differences against a saturated calomel electrode were then made, saturated potassium chloride solution being the bridge liquid and a Leeds and Northrup Type K potentiometer the measuring instrument. Then pH values were calculated from the mean of such readings as checked within approximately a millevolt.

A few drops of the same extract were saturated with quinhydrone and drawn into the capillary electrodes. The electrode wires were of pure gold B&S gauge No. 19, welded to platinum wires, B&S gauge No. 21, which passed through the glass support and established connection with copper wires leading to the potentiometer. These electrodes were cleaned every few days by removing the surface electrolytically and plating with gold from a cyanide solution. Readings were made as with the Bailey hydrogen electrode, but with switches reversed, as the quinhydrone electrode is positive with respect to the saturated calomel electrode at pH values less than 7.66.

For the other method, which we have called the ball quinhydrone method, the sample was prepared by rubbing in a glass mortar 3 to 5 grams of crumb with sufficient quinhydrone to color the mass gray black. Whenever the crumbs did not cohere, a few drops of water were added and mixing was continued until a stiff dough was obtained. With fresh crumb, water was usually unnecessary; with air-dried crumb, never more than one cc. of water was required for 5 grams. An excess of water makes a pasty dough that will not cling to the electrode. Portions of the mass were then pressed on the same gold wire electrodes that were used in the capillary method, the glass sleeves being omitted. The balls of crumb were shaped into "cocoons" about 2.5 cm. long by 1.0 cm. in diameter, each wire extending about 2.0 cm. longitudinally

into its "cocoon." The lower portion of the electrode wire was thus completely covered with a 0.5 cm. layer of sample. After about 60 seconds standing to insure equilibrium between samples and electrode, connection was made to the potentiometer, the tip of the ball was allowed to touch the saturated potassium chloride bridge, and the potential difference was read. Only readings checking within approximately a millivolt were used for calculating pH values. In neither of the quinhydrone methods was the proportion of quinhydrone used determined, nor was the use of platinum electrodes attempted.

Experimental Results

Several types of cake and bread were used as samples for comparison of the three methods of determining pH values. The basic formulas were varied in several ways in an attempt to determine what ingredients caused the variations in the differences found. The concentrated sour skimmilk used in several of the samples has been developed in these laboratories and is now being tested as to its suitability for use in a considerable number of bakery products. Comparisons were also made on two flour samples—one unbleached, the other chlorine-bleached. Table I gives the basic formulas of the cakes and baking powder breads. The yeast breads contained in each loaf 325 g. flour, 10 g. sugar, 5 g. salt, and 10 g. yeast. Sufficient water was used to give the desired consistency to the dough. The fermentation was at 28°C. for 2 hours. Tables II and III show variations in composition of the products tested, the pH values obtained, and the differences between the results for each combination of the two methods.

TABLE I
FORMULAS FOR CAKES, BISCUITS, AND MUFFINS

Component	Chocolate cake formula 1	Plain cake formula 2	White cake formula 3	Angel cake formula 4	Sponge cake formula 5	Biscuit	Muffins
	grams	grams	grams	grams	grams	grams	grams
Butter	53	70	50			68	40
Sugar	200	200	122	150	100		25
Egg yolk	36	36			45		18
Egg white	60	60	55	114	75		30
Flour, soft	128	174	126	48	48	256	216
Baking powder		10	3			12	12
Soda	variable						
Cream of tartar				1½	1		
Salt	1	1	1	½	¼		
Whole milk		163	82			174	244
Water	158				22		
Chocolate	57						
Conc. sour skimmilk	31						

The most striking features of these results are the magnitudes of some of the differences observed, the fair degree of consistency among

the differences for any single type of product (with the interesting exception of the No. 2 series of yeast breads), and the fact, which is in a measure a corollary of the preceding one, that any one of the three methods gives practically the same degree of differences between any two products of the same type. The No. 2 series of yeast breads, with which may also be considered No. 1a altho it was of a different baking, is consistent in its apparent inconsistency, since the differences between the results by the Bailey hydrogen method and by each of the two quinhydrone methods vary directly with the amount of sodium bicarbonate present, and the differences between the results by the two quinhydrone methods show a nodal rise and fall with increasing sodium bicarbonate. In other cases the effect of single constituents on the differences is not so evident.

TABLE II

COMPARISON OF pH VALUES OBTAINED BY THE BAILEY HYDROGEN, THE CAPILLARY QUINHYDRONE, AND THE BALL QUINHYDRONE METHODS

Cakes and baking powder breads

Laboratory No.	Basic formulas and variations*	pH values			Differences in pH values		
		Bailey hydrogen	Capillary quinhydrone	Ball quinhydrone	Bailey minus capillary	Bailey minus ball	Capillary minus ball
		pH	pH	pH	pH	pH	pH
1	1. 3g. soda	7.23	7.08	6.75	0.15	0.48	0.33
2	1. 1½g. soda	5.98	5.98	5.67	0.00	0.31	0.31
2	As above, 2nd determination	5.99	5.98	.....	0.01	.....	.....
4	1. 3g. soda, diff. choch.	7.35	7.09	6.85	0.26	0.50	0.24
5	1. 3g. soda, no salt	7.32	7.09	6.83	0.23	0.49	0.26
7	1. 3g. soda, 3g. T. B. P.	7.33	7.22	6.89	0.11	0.44	0.33
9	3. 3g. T. B. P.	6.63	6.67	6.11	−0.04	0.52	0.56
10	3. 3g. T. B. P., 16g. CSSM	5.53	5.60	5.27	−0.07	0.26	0.33
11	4. 1½g. C. T.	6.29	6.45	6.11	−0.16	0.18	0.34
12	5. 1g. C. T.	6.15	6.18	5.86	−0.03	0.29	0.32
13	2. 10g. APBP	6.97	6.98	6.38	−0.01	0.59	0.60
14	2. 10g. APBP, 1½g. CT.	6.53	6.51	5.88	0.02	0.65	0.63
15	2. 12g. APBP, no egg yolk	7.43	7.32	6.66	0.11	0.77	0.66
16	Muffins	7.19	7.16	6.51	0.03	0.68	0.65
17	Biscuit	7.08	7.14	6.51	−0.06	0.57	0.63
18	Soda cracker, purchased	7.42	7.21	7.02	0.21	0.40	0.19

*See Table I. TBP—Tartrate baking powder; CSSM, Concentrated sour skimmilk; CT, Cream of tartar; APBP, Alum phosphate baking powder.

Altho other details could be obtained from a study of Tables II and III, for the purposes of this paper only those facts are of interest that bear on the reasons for the differences found between the results of the methods and that, consequently, should determine under what conditions each method might properly be used. Therefore several other pertinent experiments will be considered before proceeding to discussion.

TABLE III
COMPARISON OF pH VALUES OBTAINED BY THE BAILEY HYDROGEN, CAPILLARY QUINHYDRONE, AND BALL QUINHYDRONE METHODS
Flours and yeast breads

Laboratory No.	Variations	pH values: Bailey hydrogen	Capillary quinhydrone	Ball quinhydrone	Differences in pH values: Bailey minus capillary	Bailey minus ball	Capillary minus ball
		pH	pH	pH	pH	pH	pH
Flours							
A	Unbleached	6.20	6.31	5.72	−0.11	0.48	0.59
D	Bleached with chlorine	5.67	5.79	5.29	−0.12	0.38	0.50
Breads							
1a	No alkali added	5.22	5.35	5.19	−0.13	0.03	0.16
1b	25 cc. N/10 NaOH	5.44	5.63	5.45	−0.19	−0.01	0.18
1c	90 cc. N/10 NaOH	6.51	6.67	6.48	−0.16	0.03	0.19
1d	20 g. CSSM*	4.53	4.61	4.50	−0.08	0.03	0.11
2a	45 cc. N/10 $NaHCO_3$	5.83	5.96	5.68	−0.13	0.15	0.28
2b	90 cc. N/10 $NaHCO_3$	6.63	6.64	6.22	−0.01	0.41	0.42
2c	135 cc. N/10 $NaHCO_3$	7.62	7.12	6.85	0.50	0.77	0.27
2d	180 cc. N/10 $NaHCO_3$	8.22	7.48	7.41	0.74	0.81	0.07

*CSSM — Concentrated sour skimmilk.

It was thought that the effect on the pH values of the differences in technic of preparation of the samples for each method should be investigated before a fair comparison of the methods themselves could properly be made. Extractions were carried out on two cakes for 30 minutes and for 60 minutes, 20 g. of crumb being used to 100 cc. water. Determinations of pH values were made on each extract by both the Bailey hydrogen and the capillary quinhydrone methods. On the 30-minute extracts, additional determinations were made after they had been allowed to stand for an additional 30 minutes. No differences greater than the allowable limit of error were obtained, which indicates that the time of extraction is not a critical factor.

Whether the ball method is sensitive to possible variations in moisture was determined by adding several measured volumes of water to weighed amounts of dry crumbs before determining the pH value. The data and calculated results are given in Table IV. It was difficult to make the unmoistened sample cling to the electrode, the sample with 1.50 cc. of water for 5 g. of crumb was more sticky than is convenient, but the other samples were satisfactory. The variations are all within limits of allowable error. The potential differences are included in this table to illustrate to what degree the readings by this method are reproducible.

TABLE IV

Effect of Addition of Small Amounts of Water to Cake Crumb on Results Obtained by the Ball Quinhydrone Method for Determining pH Values

Cake No. 7

Weight of crumb	Volume of water added	Potential difference	Mean potential difference	Temperature	pH value
grams	cc.	volts	volts	°C.	
		0.0439			
5.00	0.00	0.0440	0.0439	26.0	6.89
		0.0439			
		0.0438			
		0.0429			
5.00	0.50	0.0432	0.0431	26.0	6.90
		0.0429			
		0.0433			
		0.0436			
5.00	1.00	0.0428	0.0430	26.0	6.90
		0.0426			
		0.0431			
		0.0431			
5.00	1.50	0.0432	0.0434	26.0	6.90
		0.0438			
		0.0435			
Not	Not	0.0428			
weighed	measured	0.0431	0.0430	27.0	6.89
		0.0432			

Whether the addition of water in the preparation of the extracts does change the actual H-ion concentration (or activity) was investigated by comparing values obtained by the appropriate quinhydrone methods on the crumb, the extracted crumb, and the extract from the crumb. For one cake, the ball method gave pH values of 6.86 for the crumb and 7.13 for the extracted crumb; the capillary method gave 7.28 for the extract. For another cake the values were 6.72 for the crumb, 7.22 for the extracted crumb, and 7.38 for the extract. The significant facts here are that evidently the H-ion concentration was changed by the extraction process and that the change was due not merely to a dilution effect on the substances in solution, as the extracted crumb had a pH value approaching that of the extract.

An experiment that is more conclusive gave the results shown in Table V. Suspensions were prepared with different ratios of crumb and water and the pH values determined by the capillary quinhydrone method. Determinations were also made on extracts when possible and a pH value on the dry crumb was obtained by the ball method. It will be seen that each successive increase in dilution increases the pH value by successively decreasing amounts, which approach the limit of accuracy with the last increment used. Results of Johnson and Bailey (1924) show only slight changes in pH values of cracker meal extracts between

concentrations of 20 g. to 100 cc. and 20 g. to 200 cc. of water. Halton and Fisher (1928) found that "H-ion concentration of flour extracts increases as the ratio of flour to water increases," and that the curves of H-ion concentration plotted against dilution for two flours may actually cross. A calculation from the data of Table V shows that the results of Table IV should agree, as they do, within experimental error.

TABLE V
EFFECT OF DEGREE OF DILUTION ON THE pH VALUES OF CRUMB SUSPENSIONS AND EXTRACTS
EXTRACTIONS AT 25°C. FOR 30 MINUTES
Plain cake

Weight of crumb	Volume of water	pH value ball quinhydrone method	pH value capillary quinhydrone on suspension	pH value capillary quinhydrone on extract
grams	cc.			
Not weighed	00	6.67		
20	20		6.81	
20	40		7.03	7.07
20	60		7.14	7.15
20	80		7.21	7.20
20	100		7.24	7.24

In order that more convincing evidence of the ease of reproducibility of pH values by the ball quinhydrone method might be obtained, Dr. Holm and Mr. Watson, of these laboratories, kindly consented to make determinations on the samples of flour that had been used earlier. The three sets of readings on these samples were made with entirely different electrodes, potentiometers, calomel half-cells, galvanometers, and standard cells. Two of the potentiometers were L. & N. Type K, the other was the L. & N. Student Type. Only meager directions were given the two collaborators for preparing the balls of flour and neither had any information regarding the values to be expected. Three electrodes were used by Whittier, four by Holm, and two by Watson. The results are tabulated in Table VI and require no further explanation.

TABLE VI
COMPARISON OF POTENTIAL DIFFERENCES AND CORRESPONDING pH VALUES OBTAINED BY THREE DIFFERENT INVESTIGATORS USING THE BALL QUINHYDRONE METHOD ON THE SAME SAMPLES OF FLOUR

Laboratory No.	Whittier: Potential diff. against sat. cal.	Whittier: Temp.	Whittier: H-ion conc.	Holm: Potential diff. against sat. cal.	Holm: Temp.	Holm: H-ion conc.	Watson: Potential diff. against sat. cal.	Watson: Temp.	Watson: H-ion conc.
	volts	°C.	pH	volts	°C.	pH	volts	°C.	pH
Flour	0.1148			0.1149			0,1148		
A	0.1148	25.0	5.72	0.1150	24.5	5.72	0.1138	24.5	5.73
	0.1147			0.1159			0.1138		
				0.1149			0.1137		
				0.1159					
Flour	0.1402			0.1380			0.1412		
D	0.1401	25.0	5.29	0.1385	24.5	5.33	0.1405	25.0	5.28
	0.1398			0.1380			0.1412		
				0.1386			0.1409		

When the ball quinhydrone method was used and a contact of a minute was allowed between sample and electrode before taking the readings, in no case was any difficulty experienced in obtaining the degree of reproducibility shown in the tables. The capillary method in a few cases required the obtaining of a second set of three potential differences before satisfactory agreement was obtained. For the most part, it was not difficult to get satisfactorily checking readings with the Bailey hydrogen electrode, but in a few cases it was only after six or seven sets of three readings were taken that two were obtained in agreement. In all cases in which difficulty was experienced with the Bailey hydrogen electrode, the differences between the pH values calculated from the Bailey and the capillary electrode readings were strikingly large, in samples 18, 2c, and 2d, for examples. One of the authors has observed similar difficulties in the past with the Bailey hydrogen electrode.

Discussion

Whenever reproducible potential differences can be obtained at an electrode in contact with some conducting substance by any technic directed toward such measurement, it is safe to assume that one or more definite chemical or physical equilibria are determining these potential differences. This assumption is involved in any potentiometric measurement of H-ion concentration, reduction potential, flowing potential, or concentration chains. Where two or more of these factors are involved, it is obviously necessary to maintain at a constant level all the quantities except the one whose variations are being measured. With hydrogen electrodes, it is necessary to maintain the pressure of gaseous hydrogen at the electrode constant at saturation; with electrodes of the type represented by the quinhydrone electrode, it is essential that the reduction potential be maintained at a constant known value.

As the potential differences from which the pH values of Tables II and III were calculated were readily reproducible, with the exceptions previously noted, the conclusion is reached that each of these methods gives values truly representing a definite combination of H-ion concentration (or activity) and reduction level actually existing in the substances at the electrodes at the time of measurement. Since the Bailey hydrogen electrode and the capillary quinhydrone electrode values show comparatively slight tho significant differences, with the exceptions noted, it is believed that reaction between certain of the constituents of bakery products and quinhydrone or hydrogen does take place in a large number of cases, but usually to a slight degree. Differ-

ences of over 0.03 pH units between corresponding values obtained with these two electrodes apparently indicate such reaction and, as difficulty of reproduction of values appears only when such differences are conspicuously large and is confined mainly to the readings with the Bailey hydrogen electrode, it seems probable that it is the hydrogen rather than the quinhydrone that reacts with the samples.

The results in Table V, showing the change of pH values obtained by extracting crumb with progressively increasing amounts of water, furnish evidence that the differences between pH values by the capillary and the ball quinhydrone methods represent real differences between the H-ion concentration of extracts and the crumb from which such extracts are made. A number of explanations may be advanced. Dilution of the highly concentrated liquid phase may affect activities of the ions sufficiently to account for the difference or may cause a degree of hydrolysis sufficient for the result. As quinhydrone is used in both these methods, reaction of constituents of the sample with quinhydrone should not be involved in the differences observed.

For most purposes in work with flour and bakery products, investigators are interested in differences between H-ion concentration of samples rather than in absolute values. Consequently the choice of methods is usually not such a serious matter as it might at first appear. However, it is evident that results obtained by methods involving dilution should not be compared with results obtained on undiluted samples, and it must not be assumed that the H-ion concentration of an extract represents the H-ion concentration of the unextracted sample. Only in fortuitous cases would the value be the same.

In comparison with the Bailey hydrogen electrode and the capillary quinhydrone electrode, the ball quinhydrone electrode is simpler and more rapid in manipulation and its results are more readily reproducible. It is believed that the pH values obtained by the ball method are a closer approach to a true measurement of the acid-base equilibrium existing in flours and bakery products than are the values obtained by the other methods tried.

Conclusions

It is concluded from the foregoing that:

1. The H-ion concentration of a 20 gram-100 cc. extract of a flour, cake, or bread is not the same as that of the unextracted substance.

2. The Bailey electrode method and the capillary quinhydrone method applied to such extracts give results that may in general be safely used for comparative purposes, but should not be considered the true values for the unextracted samples.

3. The ball quinhydrone method, in which no added water or only a negligible amount is necessary, gives readily reproducible pH values when applied to flours and bakery products in the range 5.0 to 8.0, and the time required is comparatively short. The values so obtained more nearly represent the true H-ion concentration of the samples than those obtained by the other methods discussed.

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DETERMINATION OF DEGREE OF ACIDITY OF FLOURS BY COLORIMETRIC SPOT-TEST

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In the "maturing" of flour with "Beta Chlora" at a definite dosage, it is good mill practice to test the treated flour frequently by a very simple quick method to learn whether the desired rate of treatment is being uniformly administered. Estimation by titratable acidity, altho dependable, is too time consuming. Some method for indicating the approximate pH is helpful for this purpose. Potentiometric methods, however, are much too involved. A colorimetric method is ideally adapted to this type of control work.

The authors have found a simple colorimetric spot-test method to indicate very satisfactorily the desired maturing treatment of flours in mill control and for checking flour deliveries matured under definite specifications. Standard samples representing a series of differently matured flours are necessary for comparative purposes by this method.

The standards are prepared from flour similar to the flour under examination.

Method

APPARATUS—Porcelain spot plate (depressions of 3/8 inch depth) or small glass vials.
Eye dropper.

REAGENTS—0.02% chlorphenol red solution (pH range 5.2-6.8),
or Bromcresol green solution (pH range 4.0-5.6),
or Bromthymol blue solution (pH range 6.0-7.6).

Flour standards representing different Beta Chlora treatments, as 1.5 oz., 2.0 oz., 2.5 oz., etc., Beta Chlora respectively per barrel.

Procedure

Firmly press portions of standards and unknown in adjoining depressions of spot plate. Smooth off surfaces with spatula or "slick." Place one drop of indicator solution in center of surface of each flour portion. Let stand for three to five minutes and match spot color of the unknown with those of the standards.

The maturing treatment of the unknown sample is taken as equal to that of the standard which most closely matches its spot color. More accurate comparison of spot color shades is facilitated by covering all spots except the two under observation with two strips of white paper.

Recent observations indicate that flour freshly matured with Beta Chlora gives a spot-test indicating about 1/4 oz. per barrel less than the same flour after standing twelve hours. Matured flour after twelve hours standing apparently is stabilized and gives a constant spot-test.

The above spot-test should find valuable application wherever relative degrees of acidity of flours are of concern.

A SINGLE FIGURE ESTIMATE OF BAKING SCORES

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The importance of the experimental baking test in commercial control and in cereal research can scarcely be overestimated. Many researches have been conducted with a view to discovering some shorter means for estimating quality of wheat and flour, but the results of such work must, in nearly all cases, be referred back to the quality as determined by the baking test. It is of the utmost importance, therefore, as Blish (1927) has pointed out, to use all means possible for placing this test upon a firm scientific foundation. Important progress toward this end was made when a committee of the American Association of Cereal Chemists proposed a standard fixed procedure for use in experimental baking that practically eliminates all variables except the flour itself. A very considerable variation, however, occurs in the scoring of bread characteristics, most of which, with the exception of loaf volume, are scored customarily by the judgment of the operator.

In America, there is a general feeling that it is better to record and make use of such scores as grain, texture, color, and appearance, even tho the personal judgment of the operator is involved, than to ignore them altogether. Fisher and Halton (1929) criticize the American procedure on the ground that such scoring gives a sense of accuracy altogether unwarranted. They, however, go too far to the other extreme, as they do not record even loaf volume, but simply state their results on the basis of their judgment regarding the best loaf produced in the experiments under consideration. There is really no very distinct point of difference between these two cases because, whether the operator assigns numerical values to a number of bread characteristics and bases his opinion on these figures or whether he arrives at a conclusion by inspection of the loaf and does not record a description of it, the fact remains that the interpretation of the results of experimental baking rests entirely with those conducting the test. That the final results of a scientific test of such importance as the baking test should depend upon a factor so indeterminate as personal opinion, is indeed regrettable.

If there were available for use in recording such characteristics as grain and texture, crumb color and external appearance of the bread,

some sort of yardstick that could be applied without having recourse to individual judgment, bread scoring would consist in the recording of characteristics by means of a specified code and the method could be standardized to a considerable extent. No such system has been developed, however, and it seems likely that it will be a long time before a method entirely eliminating personal judgment is evolved. Meanwhile we must bake and must interpret the results obtained. The suggestion regarding a single-figure estimate of bread characteristics is presented with the idea of rendering more usable our present system of scoring.

If each flour tested could be baked under such conditions that the bread produced is ideal in texture, color, and appearance, the loaf volume might justly be taken as the criterion of quality; and because loaf volume can be ascertained with a considerable degree of accuracy, the results would be fairly quantitative. There would arise, of course, the difficulty of evaluating the amount of manipulation required to produce the ideal bread, and this would doubtlessly be a source of greater error than is encountered in attempting to weigh variable characteristics. In actual practice, where the procedure is varied to suit the requirements of the flour under consideration, appreciable variations in bread characteristics occur, so that these, as well as the variation in manipulation, must be taken into account in reaching an opinion regarding the quality of the flour. With a fixed procedure for all flours, irrespective of their individual requirements, many cases occur in which texture, color, and appearance are very far from the ideal. Variations of these factors are undoubtedly attributable to differences in quality, just as variations in manipulative technic necessary to produce good bread are attributable to differences in quality, and in either case the variables must be appraised in the light of experience in order to arrive at an estimate of the value of the flour for the use to which it is intended. As we have no standard system for reporting bread characteristics, it is necessary for the baker to judge or score the loaves produced, and thereby to set a value upon the various characteristics observed.

In judging bread quality there seems to be fairly general agreement regarding the importance of certain characteristics. Loaf volume is considered capable of accurate determination and is recorded in definite figures. Texture and color are usually recorded, but in less definite terms. Appearance, oven spring, crust color, and several other characteristics may or may not be scored. Doubtlessly any operator, looking over his own scoring, can form a very close estimate of the quality of the flours represented, and his scores have a very definite value for

him. To the reader examining the published data, the case is entirely different. Figures for loaf volume are more or less absolute, and may be accepted as such. The other variables are usually scored against a standard, which is taken to be 100 in all respects. It is bad enough that one has no conception of the particular characteristics of the standard, or in other words, no picture of what the author considers to be the ideal loaf, but even if one had, there is no way of ascertaining the weight of the various increments of the particular system of scoring used. In consequence of these considerations, the person examining another's baking data is inclined to rely entirely upon the loaf volume figures, being reluctant to attempt to evaluate the other variables scored. Hence, in most calculations of the coefficient of correlation between baking quality and crude protein of wheat or of flour, texture, color, and other similar scores have been neglected, and the loaf volume figure has been taken as the sole criterion of flour strength.

This attitude is perfectly justified, because there is only one person fully qualified to deduce from several more or less empirical values a single figure that might represent a sound estimate of the sum of them all. That person is the operator himself. He alone knows just how much weight the scores carry. He knows their relative value. Any one else trying to arrive at a single figure estimate would have to indulge in a considerable amount of conjecture. The adoption of a standard baking method will go a long way toward facilitating comparison of results obtained in various laboratories. It seems to be time that a further step forward be taken in order to reduce the hazard in comparing the scoring of bread characteristics.

The question of a single value to express the baking strength or baking quality of flour is by no means a novel one. The American Institute of Baking issues a score card for judging bread in which each characteristic of the bread is given an ideal value. This establishes the relative weighting of the various scores. Loaf volume is assigned a maximum of 10 points out of 100. This is in rather sharp contrast to the custom of judging quality almost wholly on volume. In experimental baking, however, much more than 10% weight necessarily must be given to volume.

Sanderson (1920) used a method for interpreting baking data into terms of price difference. He set up empirical standards as follows:

Water absorbing power	53%
Volume of loaf	2350 cc.
Color of bread when cut	95 points
Texture of crumb when cut	95 points

Deviations from these standards were given a value in terms of the price per 100 pounds of flour, and from the total could be determined the amount in cents by which the value of the flour was lowered or raised because of variation from the standard in absorption, color, texture, and volume, It is doubtful, however, that commercial value of wheat can be at all closely estimated by means of any one baking test applied to experimentally milled flour.

Saunders (1922) gave details of a system for calculating a single figure estimate of the baking score for use with experimentally baked bread. In regard to the need for some such system, he states:

> "Many years of experience have confirmed the writer's opinion that a definite scale of figures must be used to express the baking strength of flours, and that the position of any flour on this scale must be determined after considering its conduct in as many different ways as possible. The use of a fixed, numerical scale is especially important because standard flours cannot be satisfactorily employed for purposes of comparison since they themselves vary in strength with age. As we often wish to compare flours of one season with those of previous years, a shifting and variable standard is out of the question. The great difficulty, however, is to decide just what sort of a scale to establish and what importance to attach to each different observation in order to determine the place on that scale of any sample of flour which has been baked."

In computing the value for baking strength, he takes cognizance of the following variables: Water absorbed, water retained, loaf volume, height of loaf divided by diameter, form of crust, texture, and inside color. The different values for these characters are weighted empirically and a single number is obtained from the whole aggregate. A specimen calculation by Saunders' method is given below.

Water retained x 1	40
Volume of loaf x 1	526
Form of crust x 1	85
Water absorbed x 3	180
Texture x 3	264
Shape of loaf x 500	320
Total	1415

Divide by 10 and subtract 46 from the dividend.

$$\frac{1415}{10} - 46 = 95.5 = \text{baking strength}$$

Whether or not one agrees with this particular weighting, I think it will be agreed that Saunders' single figure estimate of baking strength gives a more accurate measure of flour than could be obtained by inspection of his gross data. The one who scores the bread is best

equipped to determine the degree of importance attached to each figure tabulated. Anyone else is liable to fall into grave error in trying to weight them. If, on the other hand, texture and color scores are disregarded entirely and loaf volume alone is taken as the criterion of baking quality, large errors certainly must be encountered. The soundness of making comparisons using loaf volume only may be judged from the data presented in Table I. These data were chosen from a large number of baking tests conducted in this laboratory, and were selected to show what variations, particularly in color and texture, may be found in loaves of the same volume score.

TABLE I

BAKING DATA ON VARIOUS FLOURS WITH COMPUTED BAKING SCORE CALCULATED BY THREE METHODS

Flour No.	Absorption %	Appearance	Crumb color	Texture	Loaf volume cc.	Computed baking score		
						Method I	Method II	Method III
				Group 1				
1	68	10	8	10	620	105	108	100
2	70	10	7	7	620	95	99	89
3	69	10	5	4.5	621	86	85	78
4	73	8.5	3	4	620	81RC*	83	70
				Group 2				
5	60	10	10	10	590	104	98	98
6	63	10	10	9.5	588	102	99	96
7	64	10	9	8	595	98	95	91
8	67	10	7	7.5	592	94	92	85
9	62	10	7	6.5	587	90	83	81
10	68	9	6	5	595	85	83	75
11	67	7	6	4.5	597	83	79	72
12	66	10	6	2	593	76RT†	73	67
13	74	10	3.5	3	597	77RC	79	65
				Group 3				
14	61	9	9	10	545	98	87	86
15	65	8.5	9	8	543	92	84	79
16	62	7	8.5	7.5	546	89	78	76
17	61	8	8	7	545	88	75	74
18	63	8	7	6	550	84	73	70
19	66	9	5.5	5.5	550	82	72	67
20	61	7	8	4.5	544	79	66	65
21	67	10	4	6	540	81RC	71	64
22	66	8	6.5	5	542	80	70	64
23	68	10	4	5	545	78RC	70	62
24	65	10	4	5	542	78RC	66	61
25	64	10	4	3.5	550	78RC	62	58
				Group 4				
26	62	8	9	8	490	86	70	68
27	59	10	8	7.5	493	85	66	67
28	62	8	6	9	495	86	68	66
29	57	8	6	6	499	78	55	58
30	71	10	4	5	496	74RC	63	52
31	72	9	3.5	4	494	69RC	59	47
32	75	6	2	2	492	60RCT‡	49	34
33	67	6	4	1	492	59RCT	42	35

*RC means Rejected on account of color.
†RT means Rejected on account of texture.
‡RCT means Rejected on account of both color and texture.

In baking, the Werner formula was used, with a procedure fixed in every respect except absorption. With flours varying from 57% to 75% absorption (on the 13.5% moisture basis) it is unwise to attempt a fixed absorption. Mixing was done by means of a Hobart mixing machine, which has been found to work very satisfactorily with 100 grams of flour. Fermentation and proofing were carried out as outlined by Blish (1928). The loaves were baked in low tins of dimensions given by Herman and Hart (1927) and measured in a device similar in design to the one described by Geddes and Binnington (1928).

Here, as in most research laboratories, we have occasion to deal with flours that represent the whole range from excellent to rejected. Hence in scoring texture, color, and appearance one finds frequently side by side a loaf of excellent quality and one practically unfit for human consumption, or at least unsalable. Evidently in such a case one's values must begin at 0. The question then arises: How far up is it practical to go? To carry the scale up to 100 would mean dividing the range from rejected to excellent into 100 parts. This seems likely to lead to large errors. In practice, most laboratories using this scale seldom report values below 88, and this means that the effective range is 13 points. A texture score of 88 often indicates very poor texture, but it leaves room for the inference that it is only 12% lower in merit than the standard, and this, in most cases, is quite erroneous. With descriptive terms such as excellent, good, fair, and poor, only about 12 categories are practicable. Descriptive terms, however, must be interpreted into numerical terms before they can be used in calculations. We have assigned values to the various types of texture, color, and appearance, and use a range from 0 to 10, allowing 10 to represent the maximum value of the score for the best experimentally milled flours matured by natural ageing, and 0 the value for the score when the bread is so poor as to be considered unfit for consumption. The fractional values appearing in the table occur as a result of averaging replicates. Variations in scoring replicates baked on different days are usually not greater than one point. In practically all the baking in this laboratory, the replicates are baked on different days and frequently by different bakers. Averages obtained in this way are considered fairly reliable.

In order to achieve any degree of uniformity in bread scoring, it is necessary to use a standard flour that gives consistent results. Frequent changing of standard leads to variations in scoring, unless the variations in the standard itself are scored against a selected flour which measures up to definite requirements. It seems desirable to

use, if possible, as a top standard, a flour that will produce bread of high quality. Frequently, however, the standard flour used does not produce bread of ideal characteristics, and in such cases there is no good reason for assigning a score of 100 or, in our usage, 10, simply because the flour happens to be the standard. If Standard No. 2 happens to be inferior in texture or color to Standard No. 1, it should be scored accordingly, and subsequent comparisons should be based on those scores. In lieu of a perfect scoring system, this would be an approximation to an absolute basis.

Using a top standard, it is not difficult to judge accurately the better quality breads, but it is difficult to obtain consistent scoring for the poor ones, particularly in regard to texture. It is therefore desirable to use a low quality standard, the scores for which have been carefully appraised. The need for this is greater in laboratories testing experimentally milled flours than in control laboratories testing commercial flours, altho in the latter two standards might prove useful at certain times.

Turning now to a consideration of the data of Table I, it is readily apparent that loaf volume is inadequate as a means for estimating the merits of the respective flours represented. The data have been arranged into four groups, such that the variation in loaf volume for any given group does not exceed 10 cc. They are chosen from actual cases encountered during the course of four months. Even a cursory examination of this table will leave little doubt regarding the value of the loaf volume score alone. Nos. 1 and 4, for instance, are not at all in the same class, altho they both show the same volume. By no stretch of the imagination could they be considered even nearly equal in baking value. This is one of the simplest cases, and it presents no great difficulty of judgment to place the cases in their proper order.

Samples such as Nos. 21 and 22 are more difficult to deal with. No. 22 has a lower texture score, higher color score, and lower appearance score than No. 21. If differences in these characteristics of the bread are any reflection of differences in the flour, and it seems highly probable that they are, some means must be found to evaluate the variation. When all the scores vary, the task of estimating the net result of the baking test becomes still more difficult. Samples Nos. 4 and 19 vary in every characteristic; No. 4 is scored higher in loaf volume but lower in texture, color, and appearance, and the ques ion to be settled in comparing them is whether or not the differences in the former offset the differences in the latter. This can be decided only by use of some arbitrary method of calculation.

Any method for calculating a single figure to represent the baking performance of flour, by any given procedure, must be more or less empirical. In order to be useful it should be simple and rapid and should give a range of values large enough for ready classification without having to take into account differences that might be attributable to experimental error. It is desirable, moreover, to choose a system that will give values close to 100 for very good flours and low values for poor flours, especially if such figures are to be used for the information of the general public.

The weight attached to each variable will depend to a large extent upon the experimenter's faith in the accuracy of his scoring. It will also depend upon the importance he attaches to each characteristic recorded. Saunders (1922), for instance, gave the shape of the loaf a weight equal to over 60% of the loaf volume, while to texture he assigned a weight of approximately 50% of the loaf volume. But he used low-sided, round tins, which permitted the weak flours to spread and run out, and thus this value became very important. He weighted "water absorbed" nearly 25% of volume, but this would lead frequently to very erroneous results, because wheats heavily damaged, particularly by frost, give flours having an extraordinarily high absorption. Abnormally high absorption usually indicates very low quality, and if this factor were given a weight equal to 25% of the volume it might in some cases nearly offset all the other deficiencies of the flour. "Water retained in the bread" tends to give erroneous results in a calculation of "strength," because heavy, soggy bread usually retains more water than bread of high quality. Consequently the variables scored in this laboratory are volume, texture, crumb color, and outside appearance of the loaf. This is, in fact, a general practice in many laboratories.

One method employed for computing a figure to represent the aggregate of these variables, referred to as "computed baking score," may be described as follows: The texture score is so weighted that one point in the actual score is equivalent to 30 cc. in loaf volume. Crumb color is weighted approximately one-third as heavily as texture, so that one point in color has as much effect on the final figure as 10 cc. in loaf volume. Outside appearance carries a weight such that one point in this score affects the final figure to the same extent as 5 cc. difference in loaf volume.

This may be seen more clearly from an example. Using the data for flour No. 1, Table I, the calculation is as follows:

Loaf volume	620 x 0.1	=	62
Texture	10 x 3	=	30
Crumb color	8 x 1	=	8
Appearance	10 x 0.5	=	5
Total			105

Outside appearance, in which is included crust color and symmetry, carries the least weight, because with few exceptions these factors may be readily modified. In most cases, too, where shape and color are poor, there is an accompanying heavy scoring down for texture. The exceptions mentioned above give an appearance described by the committee on standardization of the experimental baking test (Blish 1928) under Types G and H. Such loaves frequently have very good texture. It is our practice to score the appearance of loaves of this sort very low.

Crumb color in bread baked from experimentally milled flours should carry considerable weight as it indicates to some extent the degree of treatment necessary to produce a good salable product. On the other hand, it must not be weighted too heavily, as it usually can be changed effectively without materially altering the quality of the flour.

Texture, in which term is included "grain," "texture," and "resiliency of crumb," is weighted heavily, because, next to volume, it is the most important characteristic of the loaf. Perhaps it is not given enough importance in this calculation. The difficulty here, however, lies in the fact that one point in texture score is equivalent to 30 cc. in loaf volume, as far as the final figure is concerned, and this would make possible very large errors when a single bake is used. However, by the method of running replicate bakes on different days, the error from this source is considerably reduced, as the average scores are used in the final computations.

Scores for baking quality of 100 or over indicate excellent bread, or flour of exceptional strength; scores of 90-99 are given by strong flours; 80-89 by flours of fair or medium strength; 70-79 by flours of poor or low strength; while scores below 70 are given by very poor flours. In Table I, the computed baking scores shown under the heading "Method I" are calculated by this method.

The chief criticism of this method is that it gives a range of insufficient length. All types of flours from poor to excellent would fall between 70 and 110, largely because in dealing with the volume score a constant quantity amounting to about 35 points is carried into the final score in each case.

In this laboratory, a flour giving a loaf volume less than 350 cc. has rarely been baked; few have been below 400 cc. As in the other scores the range is from zero up, it seems reasonable to use a similar range for loaf volume. This may be achieved by subtracting a fixed figure from the volume obtained. In that case volume would be scored on differences from an arbitrary minimum, and this would have the effect of increasing the range of values obtained for the computed baking score.

The baking scores shown in Table I under the heading "Method II" were calculated as follows, using No. 1 as an example:[1]

(Loaf volume — 400) x 0.2	220 x 0.2	= 44
Texture x 3	10 x 3	= 30
Crumb color x 2	8 x 2	= 16
Appearance x 1	10 x 1	= 10
(Absorption — 60) x 1	8 x 1	= 8
Total		108

This method gives more weight to loaf volume differences and to color than Method I and also includes absorption. The calculations are made on differences of the variables from an arbitrary zero or minimum. The weighting of the various factors is seen from the following equalities in terms of final score:

30 cc. loaf volume
2 points in texture
3 points in color
6 points in appearance
6 points in absorption

} are each equivalent to 6 points in baking score.

While the actual weighting of loaf volume appears to be lower than in Method I, differences have twice the effect on the baking score. Crumb color is given greater weight in order to provide means for more severe penalizing of flours of objectionable color, as this is a very important consideration, especially in comparing wheat varieties. An absorption of 60% is considered to be as low as a good normal flour should show, and anything higher is considered an advantage. This does not strictly hold true, however. Wheat heavily damaged by frost gives flour of very high absorption, but this can scarcely be considered an advantage because usually the abnormally high water capacity is associated with exceedingly low quality. Frequently in such cases the increment due to absorption partly offsets the discounts made on account of poor texture and color. Such cases are, however, exceptional, and in general it is considered that the higher absorption should be recognized as a factor of quality to be credited to the flour.

The values shown under the heading "Method III," in Table I, are computed by Method II, omitting the absorption altogether. This method is applicable to the strictly fixed procedure in which the water added is a constant amount.

One fault common to all three methods is that they provide no means for adequately degrading a flour showing an unacceptable deficiency in one characteristic and good in all other respects. In Nos.

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4, 13, 23, 24, 25, 30, and 31 the color is poor enough to condemn the wheat for use in manufacture of domestic flour. In musty wheat, which gives a bad odor, and heavily sprouted wheat, which gives a sticky texture, volume may be high, but the wheats should be classed as rejected. The baking score as computed does not degrade them sufficiently. To overcome this difficulty, a limit for acceptability is set for the factors crumb color and texture; in the former, anything scoring below 5, and in the latter anything below 3 is considered rejected on either account, and this is indicated by making the notation against the baking score, i. e., RC meaning rejected on account of color, RT meaning rejected on account of texture.

The particular value of a single figure to express the total baking score is that it gives in very concise form the operator's estimate of the characteristics of bread produced from a given flour by any particular method. Any one examining the data of Table I would recognize the fact that in any group wide variations exist, but it would undoubtedly be somewhat difficult to sum up the five variables scores and to arrive at a conclusion that would coincide with that of the one who did the scoring. Baking data would be more useful if they were accompanied by some figure expressing the operator's final conclusion in regard to the individuals under examination. Whether or not such figures are to be accepted as the final expression of quality of the flours is another consideration. We have been concerned in this paper only with bread characteristics and means for rendering the scoring of them more intelligible and useful.

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MODIFICATIONS OF RUMSEY'S METHOD FOR THE DETERMINATION OF DIASTATIC ACTIVITY IN FLOUR[1]

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Introduction

The influence of diastatic action on the bread produced from any flour is well known. While it is true that the use of malt preparations has somewhat lessened the importance of the natural diastatic activity from a commercial point of view, a moderately high diastatic activity is still sufficiently desirable to warrant further investigation of this aspect of flour quality. The method most generally used by cereal chemists for the determination of diastatic activity is that proposed by Rumsey (1922). This paper outlines some modifications of Rumsey's method that have been adopted at the University of Alberta.

Control of Acidity

Rumsey found that diastatic activity varied greatly with temperature and accordingly specified in his method that the temperature of digestion should be exactly 27°C. He also found that diastatic activity was profoundly influenced by the acidity of the flour suspension, the maximum being reached about pH 4.7, but he did not recommend the control of this variable. Sorenson (1924) pointed out the impossibility of obtaining comparative results at varying hydrogen-ion concentration. He suggests that the ideal procedure is to make determinations at a number of different acidities. However, it appears to the author that where this procedure is not practical on account of the labor involved, it is desirable to make all determinations in the region of the optimum pH. The easiest method of controlling the acidity is to use a buffer solution. It was necessary, therefore, to find a buffer solution that would give the requisite pH and that was made up of substances which would neither stimulate nor depress the activity of the diastase apart from their effect on acidity. One of Sorenson's citrate–HCl buffers (I.C.T. Vol. 1) (citrate solution 8 parts, 0.1N HCl 2 parts)[2] was finally chosen. In view of the fact that flour is

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[1] Abstracted from a thesis presented to the faculty of the Graduate School of the University of Minnesota in partial fulfilment of the requirements for the degree of Doctor of Philosophy, in August, 1928.

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[2] Citrate solution — 21.008 g. crystalline citric acid + 200 cc. N/1 NaOH per litre.

itself a buffer, it was necessary to determine by experiment whether the desired pH was being obtained. Flour suspensions were made up with 10 grams of flour in 100 cc. of water or of buffer solution (1 part buffer to 3 parts water) and allowed to extract for 20 minutes. The liquid was then decanted off and the acidity determined electrometrically. The results are given in Table I.

TABLE I
pH of Flour Suspensions

Sample	Unbuffered	Buffered
1	6.00	4.63
2	5.83	4.65
3	5.93	4.63

These acidities are sufficiently close to pH 4.7 to bring the diastatic activity into the flat portion of the pH activity curve lying on each side of the maximum.

It remained to determine whether or not sodium citrate had any effect on the diastase independent of the acidity of the solution.

This was done in two ways: By comparison of the activity of a buffered sample with that of one brought to pH 4.7 by use of lactic acid; and by comparison of samples where the buffer had been used undiluted and where it was used in a 3 to 1 dilution. Any effect of the sodium citrate would be expected to be more marked where the buffer was used undiluted. The samples were treated by the usual procedure of digesting for 1 hour, clarifying, making up to 200 cc., and determining the reducing sugars in a 50-cc. aliquot. The results, given in Table II, were corrected for the blank. A sample digested in distilled water is included to show the marked increase in diastatic activity when the acidity is controlled.

TABLE II
Effect of Sodium Citrate on Diastatic Activity

Method of controlling acidity	Maltose in 50-cc. aliquot
	mg.
None	31
Lactic acid	54
Citrate buffer (undiluted)	52
Citrate buffer (1 part to 3 parts H_2O)	53

These results show that sodium citrate has no appreciable effect on the diastase. This conclusion is confirmed by results recently obtained by Sherman, Caldwell, and Dale (1927) working with pancreatic amylase.

Correction for Volume of Flour

In Rumsey's original method, no account is taken of the volume of the flour itself when making the suspension up to volume after digestion. This introduces a slight error. Accordingly the volume occupied by 10 gm. of flour in a number of suspensions was determined. The average volume was found to be 7 cc. Therefore the practice has been adopted of allowing for this when diluting the digested suspension. The volume varies slightly from sample to sample so it is impossible to get an exact volume of liquid, but by the addition of an extra 7 cc. the resulting volume is closer to the correct value than if no allowance were made for the space occupied by the flour.

Determination of the Blank

One of the biggest sources of error in the determination of diastatic activity by Rumsey's method has been in the determination of the blank. It was difficult for one analyst or a group of analysts working with different samples of the same flour to check results. It was therefore necessary to find an improved method of determining the blank that would give more reliable results.

Rumsey's directions for the inactivation of the blank are ambiguous. After describing the procedure for inactivating the active sample and the preparation of the blank he says ". . . . immediately inhibit diastatic activity by clarifying with the sodium tungstate in the manner just described. The blank determination is then carried out in the same manner except that the addition of 0.4 cc. of concentrated H_2SO_4 is omitted on dilution to volume." In this laboratory sulphuric acid has been used in the clarification of all samples, whether blank or active, as there was no apparent reason for omitting it in the case of the blank. The following experiment shows it is fortunate that this procedure was adopted. A sample of diastase-free flour was prepared and 0.1 g. of takadiastase added to it. This was treated immediately by Rumsey's procedure, omitting the acid, and allowed to digest for 1 hour at 27° C. At the end of this time 259 mg. of reducing sugar was found. The reducing substances added in the takadiastase calculated as maltose amount to only 80 mg. It is evident that when the acid is omitted inactivation is not complete.

Preparation of diastase-free flour.—The diastase-free flour used in the foregoing experiment was prepared in the following manner: Ten grams of flour was suspended in approximately 75 cc. of water in a centrifuge tube and immediately inactivated. The suspension was made slightly alkaline, 3 cc. of 15% sodium tungstate was

run in, and sulphuric acid was added drop by drop until flocculation took place. The tube was then filled to capacity with distilled water and centrifuged. The supernatant liquid was decanted and replaced with distilled water and the mass of flour in the bottom of the tube stirred up. The tube was then shaken to ensure thorough washing and was recentrifuged. This washing procedure was repeated three times.

In order to establish the efficacy of this procedure, the flour was transferred to a 200-cc. Kohlrausch flask and suspended in 100 cc. of citrate buffer solution (pH 4.7 app.). The suspension was allowed to stand for one hour at 27° C. The sample was again subjected to treatment with sodium tungstate, centrifuged, and an aliquot taken for determination of reducing sugars by the method of Shaffer and Hartman (1921), which was used throughout these experiments.

Some difficulty was experienced, owing to the formation of a heavy foam in making the original suspension. This foam formed a solid layer on top of the liquid after centrifuging and effectively prevented a clean separation by ordinary decantation. This difficulty was obviated by two means. It was not considered safe to use caprylic alcohol as an agent for cutting the foam because of the possible effect of the alcohol on the diastase. However, it was found that if three drops of toluene, which has no effect on diastase (Sherman and Wayman 1921), are added to the water in the tube before shaking, the amount of foam is much reduced. The supernatant liquid, after centrifuging, is aspirated out through a small glass tube.

It was noticed, too, that after the second washing there was a tendency for the supernatant liquid to become cloudy. The addition of about 15 cc. of the citrate buffer solution to the wash water helps to remedy this condition.

TABLE III

Activity of Natural Diastase After Inactivation and Washing

Sample	Thiosulphate titration cc.
Shaffer and Hartmann blank	13.80
1	13.68 13.65
2	13.80 13.80
3	13.85 13.82 13.80
4	13.70 13.70

The results of all determinations agree with the blank on the reagents within experimental error. This means that there were no

reducing substances in the aliquot. The reducing sugars originally in the flour had all been removed by the washing. The natural diastase of the flour had been completely inactivated and had remained so under the conditions of digestion.

Theoretically, to be a true blank the extraction of the natural sugar of the flour should be allowed to proceed under the same conditions as the digestion. Experiments were undertaken to discover whether this procedure is sound. The first step was to ascertain whether the amount of reducing sugar in the aliquot increased with time of extraction. A series of 10-gram samples of flour were suspended and inactivated in the usual way (using acid). They were allowed to extract with occasional shaking for varying lengths of time before being made up to volume, centrifuged, and sampled for sugar determination. The time of extraction was taken as the time from suspension to sampling. The results are given in Table IV.

TABLE IV
Effect of Time of Extraction on Value of Blank

Time of extraction	Reducing sugar as maltose
min.	mgm.
10	33
30	39
60	40
90	42

It can be seen that there is a marked increase in the blank with increase in the time of extraction. This is particularly noticeable in the first half hour. This suggests that part of the variability of the Rumsey blank (using the acid) is due to differences in the time elapsing between the making of the suspension and the determination of the sugars.

The expression "marked increase" in the above discussion is used advisedly. There are cases where the diastatic activity in Rumsey units will not be over 50. It can be seen easily that an error of 7 mg. in the blank makes such results absolutely unreliable.

The next step was to determine whether there was any increase in the copper reduced, owing to the hydrolysis of the starch or of the soluble material by the acid used in inactivation. A large sample of diastase-free starch was prepared by the method outlined in the early part of this paper. This was carried through the usual inactivation procedure and allowed to stand. Portions were taken out at intervals and centrifuged and 50 cc. aliquots were taken for determination of sugars.

TABLE V
HYDROLYSIS OF STARCH FOLLOWING CLARIFICATION

Time of standing	Copper from 50-cc. aliquot
hr.	mg.
1	0.95
2	0.95
3	1.02

As was to be expected, the amount of copper reduced is negligible and none of the increase reported in Table IV can be attributed to hydrolysis of the starch.

To determine the amount of hydrolysis of the soluble carbohydrates, a large sample of flour was suspended, inactivated, and centrifuged immediately. The supernatant liquid was allowed to stand, aliquots for the determination of sugars being taken at intervals. The amounts of copper reduced are given in Table VI.

TABLE VI
HYDROLYSIS OF SUPERNATANT LIQUID ON STANDING AFTER CLARIFICATION

Time of standing	Copper from 50-cc. aliquot
hr.	mg.
0.00	8.74
0.75	8.61
1.00	8.80
2.00	9.70
26.00	17.67

It will be seen that hydrolysis does proceed but that the effect is negligible during the first hour. We must conclude, therefore, that the increase reported in Table IV is due to the increased extraction of the sugar in the flour.

Procedure for Determining the Blank in Diastatic Activity Determinations

The procedure adopted for determining the blank in diastatic activity determinations is as follows: Suspend the sample in distilled water, inactivate according to Rumsey's directions for inactivating the active sample (with acid), and allow extraction to go on for 60 minutes at 27° C. Take an aliquot and determine reducing sugars immediately. A test of this method was made by determining the blank on four 10-gram samples of one flour. The results of this test are given in Table VII.

TABLE VII
QUADRUPLICATE DETERMINATIONS OF BLANK BY ADOPTED METHOD

Sample	Reducing sugars as maltose
	mg.
1	27
2	26
3	25
4	27

These results show much better agreement than was possible with the old procedure.

Summary

1. Several modifications of Rumsey's method for the determination of diastatic activity are proposed. These are:
   a. Control of acidity.
   b. Correction for the volume of the flour.
   c. Improved procedure for the determination of the blank.
2. A method for obtaining diastase-free flour is outlined.

Acknowledgment

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WHEAT AND FLOUR STUDIES XV

THE USE OF THE VISCOMETRIC METHOD FOR MEASURING THE PROTEOCLASTIC ACTIVITY OF FLOURS[1]

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That the proteoclastic activity of flours may be an important factor in determining their quality for bread making has been recognized for many years. As early as 1884 Balland demonstrated the presence of protein-splitting enzymes in bran and in sprouted wheat flour. He used the extract from a sprouted wheat flour to prepare the dough for the ordinary gluten determination and noted that the longer this dough was allowed to stand before washing out the gluten, the less gluten was obtained. Ford and Guthrie (1908) tested the proteoclastic activity of the flours with which they worked by noting whether the flours or extracts of them were able to liquefy gelatin. They found that some flours were able to do so, others were not. In two of 12 flours, liquefaction of the gelatin occurred, and as these two flours were known to produce bread of poor quality, they ascribe the weakness of the flours to the effect of the proteoclastic enzymes in breaking down the gluten.

Baker and Hulton (1908) carried out experiments confirming those of Ford and Guthrie. Small quantities of flour were digested at 37° C. for 20 hours with water saturated with chloroform. At the end of that time the suspensions were tested for tryptophane by the Hopkins and Cole reaction. Of 9 flours tested, only one gave a strong reaction for tryptophane. This flour also yielded bread of poor quality. Baker and Hulton, therefore, attributed its poor bread making properties to the activity of the proteoclastic enzymes. When Witte-peptone was added to the flour suspensions undergoing auto-digestion, a positive tryptophane reaction was shown by all. This indicated to them that most flours probably contained ereptic enzymes; only a few contained peptic enzymes. When flours contained peptic enzymes the bread baked from them showed the deleterious effects of the enzyme on the gluten.

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Swanson and Tague (1916-17) subjected flour suspensions to auto-digestion and determined the increase in amino nitrogen at certain intervals by means of the formol titration of Sorensen (1907). They found that the presence of certain salts and organic substances affected the rate at which amino nitrogen was produced. Their data show further that only relatively small increases in amino nitrogen ocurred even tho the period of auto-digestion was extended to 7 weeks. Denham and Scott-Blair (1927) used a procedure similar to that of Swanson and Tague but made a different interpretation of the results obtained. They also found, however, that different flours differed in proteoclastic activity.

Stockham (1920) determined the proteoclastic activity of flours by observing the time required for the liquefaction of 1.5 per cent gelatin media by extracts from the various flours. Stockham's data show that, in general, the lower the grade of the flour the more rapid will be the rate of liquefaction of the gelatin. Martin (1920) observed an increase in the activity of the enzymes associated with the endosperm taken progressively from the interior to the exterior of the kernel. It is also likely that the lower grade flours contain more of the scutellum, which organ, according to the work of Mann and Harlan (1915), possesses proteoclastic enzymes that would function in a manner to liquefy the gelatin. Stockham cut wheat kernels transversely so as to separate the blossom end from the germ end and milled flour from the two lots of cut wheat. Extracts prepared from the flour milled from the germ end segments liquefied gelatin in 330 hours; the extract prepared from the blossom end flour, in 720 hours. That such proteoclastic activity had deleterious effects on the bread making properties of the flour appears from the fact that the loaf volume of bread made from flour milled from blossom ends was 2700 cc. while that of flour prepared from wheat ends containing the embryo was only 2050 cc. The texture and expansion (loaf volume minus dough volume) of the smaller loaf were also decidedly inferior to those of the larger loaf. From a study of the gelatin liquefying power of a large number of flours Stockham concluded, however, that "the differences between wheats of different classes or varieties could not be attributed, to a very great extent if at all, to proleolytic activity of the wheat."

Collatz (1922), using the viscometric procedure of Sharp and Gortner (1923), was able to show that the addition of malt preparations to flours increased their proteoclastic activity. He used the viscosity of the suspension after 5 hours digestion as the criterion of proteolysis.

Sharp and Elmer (1924) criticize the work of many of the investigators who have attempted to measure the proteoclastic activity of flours in that they have allowed the flour enzyme to act on a foreign protein and not on the native flour protein. They, therefore, allowed flour suspensions preserved with toluene to undergo auto-digestion for varying periods of time up to 5 weeks and determined the changes in the distribution of nitrogen in the fractions soluble in 5 per cent potassium sulphate solution, 70 per cent alcohol, and nitrogen not accounted for in these two fractions. They also determined the increase in amino nitrogen that occurred during the proteolysis. They hoped in this way to determine what particular proteins of the flour were most readily attacked by the enzymes. In conclusion they state that the proteoclastic enzymes of flour are capable of digesting the flour protein if given sufficient time, and that auto-digestion of the flour for considerable periods of time causes no decrease in glutenin and does cause a decrease in the gliadin and also an increase[2] in the 5 per cent potassium sulphate-soluble and amino nitrogen fractions. From these data it appears, therefore, that the albumin, the globulin, and the prolamin are most readily attacked by the natural enzymes of the flour and the glutelin is not attacked at all or at the slowest rate.

Cairns and Bailey (1928) investigated eight chemical methods for determining the proteoclastic activity of flour. Of these the formol titration method of Sorensen was found to be the most suitable. The use of the viscometric method was also investigated but they state that because of difficulties inherent in the viscosity determination as a precise measurement, the method will require further study before it will be acceptable as a basis for distinguishing small variations in the proteoclastic activity of different flours. Kent-Jones (1928), however, stated that he preferred to use the viscometric method for measuring the proteoclastic activity of flours. According to his procedure this was done by determining the rate at which flour suspensions preserved with auramine decreased in viscosity during autolysis. Cairns and Bailey also state that the amount of proteolysis occurring when suspensions of high grade flours are digested for short periods of time is small when measured in terms of the amino nitrogen which appears in the digestion mixture, and that the ash content as a measure of the grade of the flour is positively correlated with the rate of proteolysis.

[2] The increase in potassium sulphate-soluble protein obviously resulted from other flour proteins (notably gliadin) being rendered soluble in this solvent by the action of the enzymes.

Experimental

A great deal of work has been done in this laboratory (Johnson, 1927; Johnson and Herrington, 1928, 1928a) on the standardization of the viscosity test so that it might be applied to the study of the proteoclastic activity of flours. As there is no need to review this work in detail, it is sufficient only to give the procedure that was finally adopted. The proper quantity of flour of which the proteoclastic activity is to be determined is suspended in 100 cc. of toluene-containing water and the suspension placed in the thermostat. At the end of the period of auto-digestion, the suspension is rinsed into a liter Erlenmeyer flask with 900 cc. of distilled water at 40° C. The liter of suspension thus obtained is kept in a water bath at 40° C. for 45 minutes with shaking at 10-minute intervals. At the end of this time the suspension is allowed to stand for 15 minutes, whereupon most of the flour settles to the bottom of the flask and the supernatant liquid can be decanted. Another liter of water at 25° C. is then added to the precipitate and after standing for 15 minutes the supernatant liquid is again decanted. A third liter of water at 25° C. is finally added to the precipitate and after allowing the suspension to stand for 15 minutes the supernatant liquid is decanted and the residue made up to 100 cc. and poured into the cup of a MacMichael viscometer. After acidulation with 0.5 cc. of 20 per cent lactic acid, the viscosity is determined using a No. 30 wire. The cup of the MacMichael viscometer is revolved at a speed of 76 r.p.m.

In order to obtain comparable results it was found necessary to wash with three liters of water at the temperatures indicated, otherwise some of the preparations would show higher viscosities after several days digestion than they did originally. This was due to the long standing of the suspension rendering soluble larger quantities of the substances operating to decrease the viscosity so that they could be removed with fewer washings from the digested suspension than from the freshly prepared suspension. Carbon-dioxide-free distilled water was used for all the work.

To illustrate the usefulness of the method, the data will be given when it was applied to the study of the proteoclastic activity of a patent, a straight, the same straight bleached with chlorine, a first clear, and a second clear flour, all milled from the same wheat. Eighteen-gram portions were used. Their ash, protein, pH, and original viscosity are given in Table I. The original viscosities were obviously determined in the same way as were those during the course of proteolysis. The effect of temperature was also studied, the proteolysis

therefore being allowed to proceed at 20°, 25°, 30°, 35°, and 40° C. The 125-cc. sample bottles containing the suspensions were maintained at these temperatures for varying periods of time up to 5 weeks. During autolysis the bottles were not shaken, as shaking often caused erratic results. As the differences in viscosity obtained when the suspensions were shaken once each day and not shaken at all might be of interest, the data for such an experiment are given in Table II. The quantities of electrolytes removed from both sets of solutions were the same as shown by the conductivities of the decantates. The quantities of protein decanted were not determined, but it is in accordance with other observations that more protein was probably decanted from the more vigorously treated solutions. It is in line with the data of Johnson (1927), therefore, that the suspensions shaken should have the higher viscosities.

TABLE I

Protein, Ash, pH, and Viscosity of the Flours Used

Sample No.	Grade	Ash	Protein	pH	Viscosity: 12 gm. protein conc.	Viscosity: 18 gm. protein conc.
		%	%		degrees MacMichael	
1	Patent	0.41	11.35	5.78	67	226
2	Straight	0.62	11.92	6.02	72	226
3	Straight bleached	0.62	11.92	5.92	85	268
4	1st clear	0.93	12.84	6.13	114	262
5	2nd clear	1.88	13.62	6.20	54	117

TABLE II

Viscosities of Two Sets of Flour Suspensions Digested for Different Periods of Time

One set was shaken once each day, the other set was not shaken during the course of the experiment.

Duration of hydrolysis	Shaken once a day	Not shaken
days	degrees MacMichael	
1	178	158
3	135	117
5	130	111
7	112	82
14	74	47
21	66	40
28	53	28

The viscosity data obtained when the sets of solutions were autolyzed at different temperatures, are given in Table III. A study of the results shows that at any temperature a regular decrease in viscosity occurred during autolysis. This is perhaps most readily seen in Figure 1, in which the viscosities of the suspensions autolyzed at 30° are plotted against the time digested. The curves show that during the early stages of autolysis the viscosity decreased very rapidly, the rate of decrease diminishing toward the end of the digestion period.

In Figure 2 the effect of temperature on the rate of decrease in viscosity is shown. The data for the patent flour were used in drawing these curves. A similar set of curves would be obtained by using the data for any of the other flours.

TABLE III

VISCOSITIES OF EXTRACTED ACIDULATED FLOUR-WATER SUSPENSIONS OF 5 FLOURS DIGESTED FOR DIFFERENT PERIODS OF TIME VARYING FROM ONE DAY TO 5 WEEKS, AT 20, 25, 30, 35, and 40°C.

Flour	Viscosity in degrees MacMichael							
	24 hr.	48 hr.	96 hr.	1 week	2 weeks	3 weeks	4 weeks	5 weeks
				Digested at 20°C.				
1	210	180	163	127	80	65	52	46
2	197	167	126	106	55	41	22	15
3	245	208	181	142	118	80	58	52
4	215	145	109	80	45	25	8	4
5	55	40	26	23	9	......	5	......
				Digested at 25°C				
1	195	162	142	100	62	50	40	35
2	173	145	98	60	41	30	18	12
3	221	195	155	94	58	39	32	31
4	168	130	90	50	23	16	11	4
5	45	32	20	16	7	......	3	......
				Digested at 30°C.				
1	167	145	126	82	54	42	32	30
2	146	130	88	55	33	22	15	12
3	190	165	125	76	49	33	24	21
4	145	115	80	44	20	14	8	5
5	35	30	20	16	5	......	3	......
				Digested at 35°C.				
1	145	123	104	66	48	37	28	24
2	135	110	70	35	25	20	12	8
3	155	128	88	64	34	29	16	18
4	120	103	62	38	16	12	7	3
5	28	24	22	15	5	......	1	......
				Digested at 40°C.				
1	124	101	85	58	40	34	26	20
2	113	95	62	30	24	18	13	11
3	125	102	75	51	30	27	23	16
4	105	88	52	30	16	12	4	4
5	20	18	16	11	3	......	2	......

Several other methods of following the rate of proteolysis in these flours were used. The quantities of protein in the decantates obtained from washing the precipitate for the viscosity measurements were determined with the idea in mind that proteolysis would increase the quantity of soluble nitrogen. It was found, however, that in all cases except the second clear flour there was less protein in the decantates obtained after 5 weeks digestion than in those obtained when the original viscosities were determined. The phenomenon of some of the flour proteins becoming less soluble when suspended in water for extended periods of time has been noted by several investigators. It appears therefore, that the quantity of protein made insoluble by contact with water was greater than that made soluble by the action

of enzymes. In flour No. 5, a second clear flour, a gradual increase of protein in the decantates occurred that amounted to an increase of approximately 50 per cent at the end of the 5 weeks. All the available data show that the rate of proteoclastic activity was the most rapid for this flour. Johnson and Bailey (1924) have shown that changes in hydrogen-ion concentration may effect large changes in the quantity of protein dispersed in a digesting or fermenting medium. These digestion mixtures, however, were preserved with toluene and were

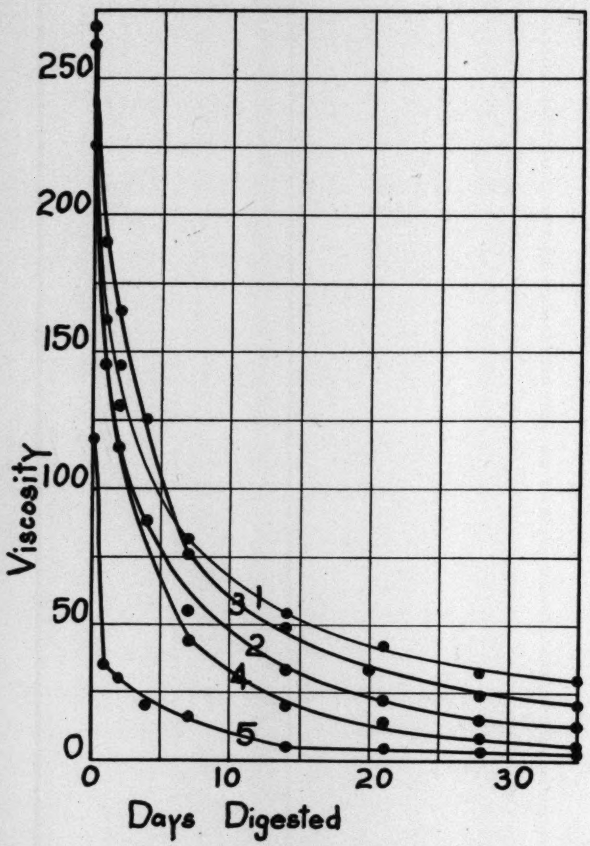

Fig. 1. Rates of Decrease in Viscosities of Acidulated Suspensions of Patent, Straight, First and Second Clear Flours During Autolysis at 30°C.

found to have approximately the same H-ion concentration at the end of the digestion period as at the beginning. Autolysis was conducted at the H-ion concentrations of the original flours, hence the rates of proteoclastic activity may not be strictly comparable. The H-ion concentration of the original flours is given in Table I. It is believed preferable, however, to investigate the proteoclastic activity of the natural flours before studying the effects of various factors.

Formol titrations were also conducted on these flours. In order to have results comparable with the viscosity data, the suspensions were prepared in the same manner, 18 grams of flour being suspended in 100 cc. of water and allowed to undergo digestion in the same way and for the same time as for the preparations used in the viscosity determinations. At the end of the digestion period the suspensions were

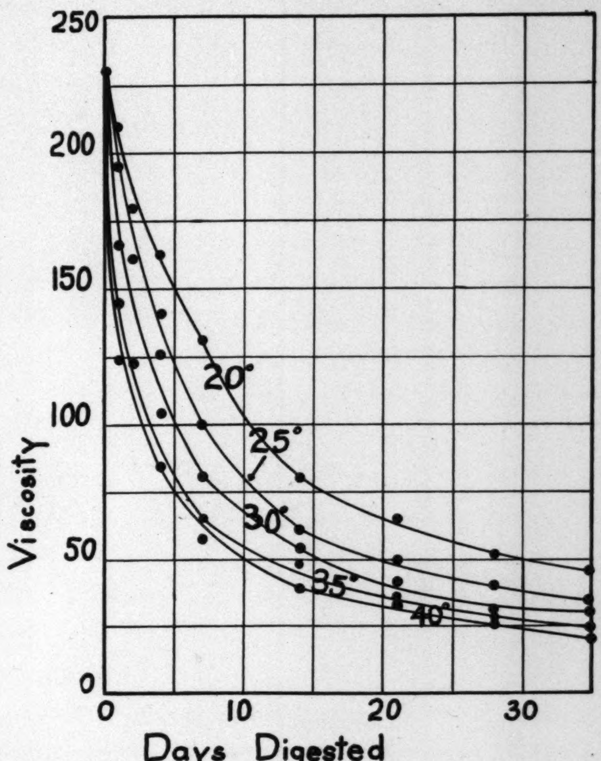

Fig. 2. Effect of Temperature of Autolysis on Rate of Decrease in Viscosity of an Acidulated Patent Flour Suspension
The temperatures of autolysis are shown on the curves.

centrifuged and 25-cc. portions used for the Sorensen formol titration in the usual way. The results were recalculated into mgm. of formol titratable nitrogen per 10 grams of flour and are given in these terms in Table IV. The results show that all the flours increased considerably in amino nitrogen content during digestion for 5 weeks. For the patent flour at 20° C. the content of amino nitrogen increased from 0.45 to 2 mgm. per 10 grams of flour, while for the second clear flour the increase was from 1.70 to 8.50 mgm. As might be expected, digestion at higher temperatures produced larger quantities of amino

nitrogen. At the end of 5 weeks digestion at 20° C., 10 grams of the patent flour produced 1.60 mgm. of amino nitrogen; at 40° C. the same quantity of flour yielded 2.50 mgm. The increase in amino nitrogen due to increased digestion temperature was considerably greater for the second clear flour; for example, at 20° C., 8.50 mgm.; at 40°, 14.20 mgm. per 10 grams of flour.

TABLE IV

INCREASE OF AMINO NITROGEN DURING AUTO-DIGESTION OF SEVERAL FLOUR SUSPENSIONS AS DETERMINED BY THE SORENSEN FORMOL TITRATION

Flour	Milligrams amino N (formol titratable) per 10 gm. flour								
	0	24 hr.	48 hr.	96 hr.	1 week	2 weeks	3 weeks	4 weeks	5 weeks
				Digested at 20°C.					
1	0.45	0.55	0.77	1.00	1.10	1.10	1.40	.....	1.60
2	0.60	0.80	0.88	1.10	1.30	1.35	1.70	1.75	2.00
3	0.65	0.80	0.81	1.10	1.40	1.30	1.70	1.85	2.10
4	0.75	0.80	1.05	1.30	1.60	1.70	2.10	2.30	2.60
5	1.70	2.35	2.60	3.90	5.00	5.80	6.40	7.90	8.50
				Digested at 25°C.					
1		0.67	0.80	0.95	1.30	1.20	1.60	1.70	1.80
2		0.80	0.95	1.20	1.40	1.75	2.00	2.15	2.40
3		0.85	1.10	1.20	1.50	1.85	2.00	2.20	2.40
4		1.10	1.20	1.45	2.05	2.15	2.80	3.00	3.10
5		2.80	3.40	4.40	5.70	7.05	7.60	8.70	10.70
				Digested at 30°C.					
1		0.70	0.95	1.00	1.35	1.50	1.70	1.90	2.00
2		0.95	1.00	1.05	1.60	1.80	2.15	2.40	2.60
3		0.80	1.05	1.10	1.45	2.10	2.10	2.40	2.60
4		1.05	1.30	1.60	1.90	2.30	2.95	3.50	4.00
5		2.60	3.60	4.40	6.10	7.60	8.45	10.80	11.80
				Digested at 35°C.					
1		0.67	0.85	1.00	1.40	1.70	2.40	2.20	.....
2		0.90	1.10	1.30	1.90	2.30	2.45	3.00	3.20
3		0.84	1.00	1.30	1.85	1.80	2.40	2.90	2.80
4		1.12	1.35	1.65	2.40	2.85	3.45	3.80	4.20
5		3.15	3.80	5.00	7.10	9.00	9.50	12.10	13.60
				Digested at 40°C.					
1		0.90	1.00	1.15	1.40	1.70	1.95	2.10	2.50
2		0.95	1.10	1.35	1.80	2.00	2.00	2.40	.....
3		0.80	1.03	1.35	1.82	1.80	2.00	2.40	2.80
4		1.30	1.45	1.60	2.30	2.75	3.30	4.00	4.50
5		3.70	3.90	5.40	7.20	8.60	9.90	12.50	14.20

The amino nitrogen content of flour suspensions that had been allowed to undergo auto-digestion for different periods of time and at different temperatures were determined also by the Van Slyke method. The flours were prepared for the digestion experiments in the same way as were those of which the viscosity and the formol titratability were measured, but at the end of the digestion period the suspensions were clarified by the use of sodium tungstate according to Rumsey's method (1922). After centrifugation, portions of the water clear solution were used in the Van Slyke apparatus. The data are given in Table V. These data again show that different quantities of amino nitrogen were

produced by the different grades of flour, more amino nitrogen being produced by the lower grades.

TABLE V

INCREASE IN AMINO NITROGEN DURING DIGESTION OF SEVERAL FLOURS AS DETERMINED BY THE VAN SLYKE METHOD

Digested in the following manner	mgm. N per 10 gm. of the flour				
	Flour No. 1	Flour No. 2	Flour No. 3	Flour No. 4	Flour No. 5
Original	0.31	0.38	0.38	0.50	1.08
5 weeks at 20°C.	0.57	0.89	0.94	1.01	3.96
5 weeks at 25°C.	0.89	1.08	1.01	1.28	4.47
5 weeks at 30°C.	0.89	1.14	1.14	1.28	5.24
5 weeks at 35°C.	1.01	1.21	1.28	1.52	6.15
5 weeks at 40°C.	1.08	1.28	1.21	1.59	6.91

The fact that the amino nitrogen content of the flour suspensions increased during auto-digestion indicates that some hydrolysis of the protein had taken place. In the patent flour digested for 5 weeks at 40° C. the amino nitrogen content determined by the formol titration method was 2.50 mgm. per 10 grams of flour. This is equivalent to 14.25 mgm. of protein, which is only about 1 per cent of the protein in the flour. It is not likely that a flour which has been suspended in water at 40° C. for 5 weeks will be fit for bread making, yet the reduction of the protein content by 0.014 gm. per 10 grams of flour would probably not substantially change the baking quality of the flour. A greater injury to the protein must therefore occur than appears from these calculations. In studying this further, the viscosity data were utilized. Gortner (1924) has shown that when the logarithms of the viscosities as ordinates are plotted against the logarithms of the flour concentrations as abscissae a straight line is obtained. Once having drawn such a line from known concentrations of flour or protein it should be possible to read off the corresponding flour or protein concentration for any given viscosity. From the viscosities at 12 and 18 gram flour-water concentrations, as given in Table I, such lines were drawn and the flour concentrations corresponding to viscosities of the digested suspensions were read from them. The difference between these values and 18, which was the flour concentration before digestion, gives the quantity of flour containing the protein of which the viscosity was reduced to 0 degrees MacMichael by the action of the enzymes. It may not be correct to consider that the proteolysis annihilated the effect on the viscosity of a definite quantity of the flour protein, leaving the rest with the same effect on viscosity as before autolysis. It will amount to the same thing in the end, however, whether one assumes that the effect of a part of the protein on viscosity has been completely destroyed or that the effect of all of the protein has been

reduced; that is, the effect of the autolysis on viscosity will be equivalent to a certain quantity of original flour protein. This idea must be kept in mind in interpreting the data.

In Table VI the quantities and percentages of flour no longer operating to increase the viscosity of the acidulated suspensions are given for the 24-hour digestion period. It was judged unnecessary to read off values for longer digestion periods, as in ordinary bread or cracker making the flour does not remain in the dough form much longer than this.

TABLE VI

Quantity and Percentage of Flour in 18-gram Sample that no Longer Operated to Increase the Viscosity When the Suspension was Acidulated

Digested for 24 hours at the following temperatures, °C.	Quantity of flour no longer operating to increase viscosity				
	Flour No. 1	Flour No. 2	Flour No. 3	Flour No. 4	Flour No. 5
	gm.	gm.	gm.	gm.	gm.
20	0.4	0.8	0.6	1.7	5.9
25	0.7	1.6	1.4	3.5	7.6
30	1.7	2.6	2.1	4.5	8.5
35	2.5	3.0	3.1	6.0	9.5
40	3.3	4.0	4.3	6.6	11.0
	Percentage of flour no longer operating to increase viscosity				
20	2.22	4.44	3.33	3.89	32.77
25	3.89	8.88	7.77	19.44	42.21
30	9.44	14.44	11.66	25.00	47.21
35	13.88	16.66	17.22	33.33	52.77
40	18.33	22.22	23.88	36.67	61.11

The data in Table VI show that the viscosity of relatively large quantities of flour is reduced to 0 degrees MacMichael by the action of the natural enzymes of the flour during 24 hours. At 30° C. the 18-gram patent flour suspension digested for 24 hours has a viscosity corresponding to 16.3 grams of fresh flour. This indicates that the effect on the viscosity of 1.7 grams, or 9.44 per cent, of the flour has been reduced to zero. For the lower grade flours the effect is much greater. In the second clear flour at 30° C. the effect on the viscosity of 47.21 per cent of the flour has been destroyed. This percentage is also the percentage of the protein of which the effect on viscosity has been reduced to 0 degrees MacMichael. Auto-digestion of the 5 flours at 40° C. for 24 hours, accordingly reduced to 0 the effects on the viscosity of 18.33, 22.22, 23.88, 36.67, and 61.11 per cent of the original protein. Auto-digestion appears, therefore, to effect significant changes in the physical properties of the flour proteins. As the quantities of amino nitrogen are low even after extended auto-digestion, it is evident that the magnitude of the reduction in viscosity is not reflected in the increase in amino nitrogen. It is apparent that a complete hydrolysis of the protein is not necessary to decrease the viscosity, such a decrease prob-

ably being effected by partial hydrolysis. Some investigators have suggested that the first stage of enzymic cleavage is a disaggregation of the substrate, that is, a preliminary process by which the size of the particles is rendered smaller and therefore more susceptible finally to true hydrolytic cleavage. The decrease in viscosity that occurs may be associated with such a process. This, however, does not militate against the idea that when we measure the rate of decrease of viscosity we are also measuring the rate of proteoclastic activity.

During the course of the investigation on the application of the viscometric method to the determination of the proteoclastic activity, the proteoclastic activity of a large number of flours has been investigated. For normal flours of different grades the results given are typical. Frosted and sprouted wheat flours exhibited greater proteoclastic activities than normal flours. In one experiment ground one-gram portions of heated, normal, and sprouted wheat were added to an 18-gram suspension of a check flour and the suspensions were allowed to digest at 40° C. for 48 hours, at the end of which time extractions and viscosity determinations were conducted in the usual way. The "heated" wheat was kept at 100° C. for several days in order to destroy the natural enzymes. The check flour suspension and the same, digested with heated, normal, and sprouted wheat gave viscosities of 152, 144, 101, and 55 degrees MacMichael respectively. These data show the effect on the viscosity of adding different enzyme-containing substances to the normal flour. The heated wheat probably contained no active proteases; the normal and sprouted wheats contained increasing quantities. The quantities of protein no longer operating to increase the viscosity of the acidulated suspension might be calculated in the same way as before.

Discussion

Sharp and Elmer (1924) considered the glutenin of flour to be the least readily hydrolyzed during autolysis of any of the proteins of flour. According to their method of analysis, flour suspensions digested for 5 weeks contained no less glutenin than at the beginning of the digestion period. The work of Sharp and Gortner (1923), as well as some of our own, indicates that glutenin is the protein responsible for the increase in viscosity that occurs when flour suspensions are acidulated. If this is the case, then when the proteoclastic activity is measured by the viscometric method it is really the effect of the protease on an individual protein that is being measured. This appears to be of advantage rather than otherwise, especially if glutenin is the more important protein associated with flour strength, as the work of Woodman (1922) and

Sharp and Gortner (1923) indicates. The rate of the decomposition of the most important constituent of a mixture is obviously of more significance than that of less important constituents. The change in the glutenin molecule effected by the action of the flour protease is a very subtle one as appears from the fact that the solubilities in water, in 5 per cent potassium sulphate solution, and in 70 per cent alcohol, apparently remain the same. What really happens to the glutenin when it is subjected to autolysis is an interesting problem for future study.

Perhaps the greatest disadvantage involved in the use of the viscometric method for determining the proteoclastic activity of flours is that it requires considerable time and skill. All solutions must be treated in exactly the same way both during digestion and during the preparation of the suspension for the viscosity determination. Even with the utmost care discrepancies appear. By controlling the factors that influence the viscosities, comparable results, however, can be obtained. For rapid work the formol titration has been found to give good results but, as has been shown, it gives no indication of the absolute quantities of protein changed by the hydrolysis.

The work reported in this paper has been confined strictly to a study of the activities of the proteases in flour. During bread making, yeast is added to the flour. The effect of added yeast in increasing the proteoclastic activity of flour suspensions and doughs has been the subject of a large amount of research, but there is still some doubt as to the extent to which proteolysis occurs in fermenting bread dough. It is hoped that the viscometric method described here can later be applied to this phase of the problem in such a way that more convincing results will be obtained.

Conclusions

A viscometric method of determining the proteoclastic activity of flours is described, according to which the digested flour-water suspension is extracted three times with one liter of water. The residue in a volume of 100 cc. is acidulated and the viscosity determined.

When a patent flour-water suspension is digested at 30° C. for 24 hours, about 10 per cent of the protein is changed to the extent that it no longer imbibes water on acidulation.

The effects of comparable treatment on the proteins are greater in low-grade flours. For example, 47 per cent of the protein of a second clear flour no longer inbibed water when the flour-water suspension was digested at 30° C. for 24 hours.

Rate of decrease in viscosity was greater at higher temperatures.

Amino nitrogen, as determined by both the formol titration and the Van Slyke methods, increased during autolysis of flour suspensions.

The formol titration is probably the most convenient method of determining the proteoclastic activity of flours, but information is not given concerning the absolute quantity of protein changed by the hydrolysis.

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A TECHNOLOGICAL METHOD FOR THE STUDY OF YEAST

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Ascertainment of yeast strength has been and remains of major interest, not only to the yeast manufacturer but also, if not more so, to the user of this most interesting biological commodity.

Somewhat over a year ago the authors were called upon to investigate the functional activities of different strains of pure culture yeasts. The question at issue was their respective utility for panary fermentation. It was at once recognized that the environmental variations to which yeast may be and, in fact, is inevitably exposed when performing its functions, vary from day to day, month to month, season to season, not only with geographical, climatic, and meteorological conditions, but also with the degree of control and the care taken by the baker to compensate for such variations or permitting them to predominate. Unless it be assumed, as we think it may rightly be, that different types or strains of yeasts will manifest their respective characteristics, in environments reasonably fixed, to an extent not materially changed by unescapable variations of environment, tests of the nature herein reported upon would, of course, be valueless and the problem before us would defy intelligent consideration. If we have stated here, as we hope we have, an "undisputed thing in a solemn way," we did so not for the purpose of giving information but merely to convey

that, since it is manifestly impossible to meet all variables of environment, chemical and physical, by modification of the yeast and its functional manifestations, it is necessary to make observations under conditions approaching optimum in terms of uniformity.

With substantiation, technological and scientific, and as the result of many hundreds of tests frequently verified in commercial practice, the authors firmly hold that manifested differences in functional activities of yeast under conditions favorable, will be maintained in approximately the same ratio when these conditions are varied within the limit of tolerance. If this be so, any method aiming to ascertain yeast utility for panary fermentation should therefore be made *in situ,* i.e., in the dough mass itself, and should comprise the entire process from beginning to end, and should permit of observations during the entire cycle and of any portion of it.

A test of this nature, devised for the purpose of obtaining more general information, was described by the senior author, in Cereal Chemistry, vol. II, p. 310. This test is now so well-known that detailed recital of its steps is unnecessary. It is sufficient to state that its procedure even more closely maintains the conditions which are the aim of modern regulated bakeshops. With intelligent practice and sufficient replication small differences due to change in any one material comprising the dough mass are readily studied during the process of fermentation, which fairly resembles, in its steps, modern bakeshop practice and produces a finished baked loaf. Changes in loaf characteristics such as volume, development, crust and crumb color become apparent if tangible. For the study of yeasts herein discussed, the maintenance of the chemical and physical environmental conditions of the test is, however, more essential than in the case of the mere laboratory baking test.

The desirability for testing yeasts *in situ,* appears to be well-substantiated by C. H. Bailey (*vide* the last paragraph, page 241, "The Chemistry of Wheat Flour"). Altho the authors agree that the term "diastatic activity" is not entirely appropriate, they will, for the want of a better known term, nevertheless use it and modifications of it. The terms are self-defining if it be understood that they comprise fermentable sugars in the dough mass whether due to the flour or added sugar. They stand for total available sugars.

A given yeast will behave differently when functioning in doughs of different diastastic concentration. All other physical and chemical conditions being held uniform, "diastastic deficiency" gives symptoms of age. "Diastatic sufficiency," or preferably "diastatic excess" gives

symptoms of vigor and youth. The same yeast will give on the one side pale crust color, decreased volume, even destruction of coherence in crumb and crust; and will give reverse symptoms when acting in the presence of "diastatic excess."

In the practice of the "yeast differential" test, the same procedure is followed as when making duplicate tests to determine baking characteristics of flours (See Cereal Chemistry, vol. II, p. 312). However, for the purpose of testing various yeasts the flour must of necessity be a constant. Constant flours not being an article of commerce, a standard flour must be used. It is suggested that a flour most desirable for this purpose is one which when baked for characteristics shows "diastatic deficiency" manifested by the usual symptoms of age. We have found that flours of this nature show differences between various yeasts to better advantage than flours that readily withstand the stress of the test for baking characteristics without giving symptoms of age. When testing yeast, duplicate doughs are made with a minimum or no addition of sugar on the one side (called the "negative bake") and duplicate doughs are also made with an addition of sugar, say 5% (called the "positive bake"). In the one case, under the conditions of the test procedure, we have "diastatic insufficiency," and in the other, with a proper standard flour, "diastatic excess."

If different yeasts are functioning in environments varying only in "diastatic activity," their different characteristics and manifestations are not only easy to observe but also render themselves to numerical expression.

A typical example is given below. It is selected from many hundreds of tests on different cultures. In the tabulation as well as in the cuts three drastically different yeast cultures are designated as "R," "B," and "S," respectively. They were tested with a standard flour practically neutral to the bromate differential test: ash 0.39%, protein 10.60%. In this as well as in all other tests the exact formula of the Werner laboratory baking test was used, which calls for 2½ grams of sugar per 100 grams of flour. The "positive bake" was made by adding 5 grams of sugar, 2½ grams more than was used in the "negative bake."

Yeast Culture		No. of Tests	100gm/cc.	Difference
R	Negative	16	415	
R	Positive	16	455	40
B	Negative	16	380	
B	Positive	16	480	100
S	Negative	16	355	
S	Positive	16	495	140

Figures 1, 2, and 3 picture the typical results of such a test. In each case the "negative" loaf is the unmarked loaf on top, and the "positive" loaf the marked one on the bottom. It will be noticed that in the "negative" series "R" shows the largest volume, "B" is next, and "S" the smallest. In the "positive" series the reverse is true. "R" is smallest, "B" next, and "S" largest. On the "negative" side, loaf volume descends. On the "positive" side it ascends (See Fig. 1). From these data and the external and internal characteristics of the loaf, we may well suppose that, expressed in terms of speed, "S" is the strongest yeast, "B" is next, and "R" is weakest. We may also suppose that the volume in the "negative" series is least in "S" because the sugars have been exhausted to greater extent, alcoholic fermentation has decreased, and the products that exert a degrading influence on the gluten have begun to exercise their influence. This does not happen in the "positive" series because, owing to the increased sugar concentration, alcoholic fermentation is prolonged and is not yet followed by the processes that may succeed alcoholic fermentation—processes which have a degrading influence on the gluten.

Figure 1

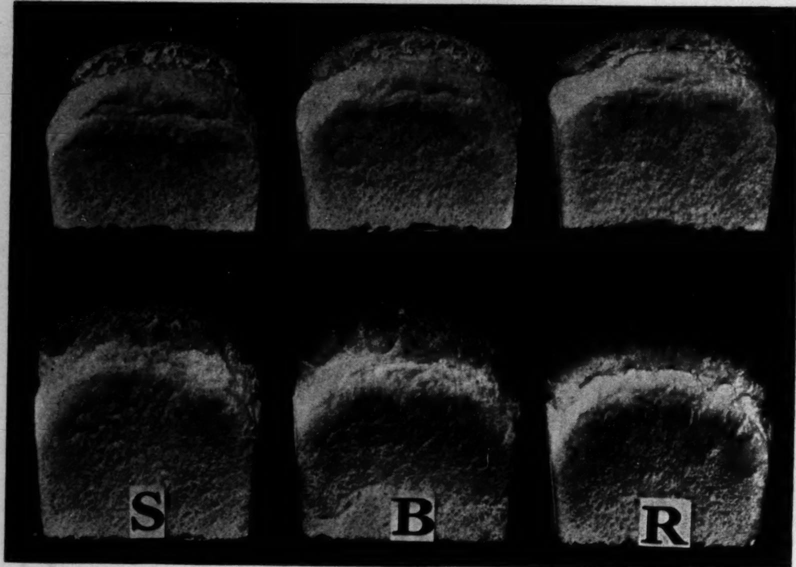

Figure 2

Figure 3

Interpretation of the data obtained by this somewhat elaborate series of experimentation apparently points in the direction of quantitative economy which might accrue from the use of cultures "B" and "S," if ever they become available in commercial quantities, over culture "R." Definite conclusions in this direction would, however, be decidedly premature and should be approached with great caution. Close observation and study of fermentation characteristics and of the external and internal appearance of the finished loaf substantiates the thought that the numerical value of "difference" also indicates, in approximately the same relation, the critical nature of the yeast strain. "R" is therefore the least, and "S" the most, critical. Type characteristics will persist; they cannot be entirely eliminated by decrease of quantity. Altho foreign to the subject matter of the present communication, the authors express the opinion that for general bakeshop practice a culture having characteristics somewhat between "R" and "B" is most desirable.

This is a test procedure to approximate the technological value of yeast strains or cultures. It is, however, obvious that the test may be modified to approximate available sugars or fermentable carbohydrates in flours. For this purpose also the authors have found this purely technological test most useful. For both purposes the test appears to be informative and to render itself to numerical expression with a degree of accuracy which has been fully established by replication.

There appears to be evidence that the (as yet undefined) term "stability" of flour may be related to yeast metabolism. On this subject the authors hope to communicate in the near future.

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GRANULATION OF FLOUR AND ITS RELATION TO BAKING QUALITY

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(Read at the convention, June, 1928)

The purpose of this investigation is to determine what effect, if any, the granulation of flour has upon its baking quality. The experiments were considered from the standpoint of bread making. This is an old subject and has been investigated many times, but it is ever recurring and apparently has not been answered to the satisfaction of all. Shollenberger and Alsberg have recently made very thoro experiments on the granulation of flour in which the effect of various degrees of grinding was carefully investigated. The conclusion drawn from the experiments is that excessive grinding is injurious and that the best flour has not too course or too fine granulation. With that as a general statement, the problem of the millers and the chemists is to find just how coarse or how fine flour should be to give best results.

In 1927-28 on the Pacific Coast we had unusual conditions that are very favorable to a study of flours produced by various mills. The hard winter wheat crop was a failure and the Northern Spring wheat raised in Washington was mostly weak and not suited for a strong bakers' flour. Consequently nearly all the strong hard wheat flours were made from Montana Dark Northern Spring, in which there was a minimum of variation. Most mills used this wheat, varying in strength from 12% to 14% protein, most of it falling within the limits of 12% to 13% protein. This wheat is one of the best grown in the United States. The soil is comparatively new and the climate is favorable for growing high quality, strong wheat. Marquis is practically the universal variety. Differences in the quality of flours from that wheat are due principally to differences in milling.

About forty samples were tested, sometimes more than one from the same mill, but at least 30 mills were represented. A granulation test and a baking test were made on each sample (see Table I).

The granulation test was made by sifting on an experimental sifter running at 180 r.p.m. with a throw of 2½ inches. Each sieve had 50 square inches of bolting surface and was clothed with bolting silk running from 10XX to 16XX. A flat rubber disk an inch and a half in diameter was placed on each sieve as a cleaner. Attached to the side of the sifter is a knocking arrangement to give a sharp tap at

each revolution. One hundred grams of flour was used for each test and the sifter was run 8 minutes. A nearly complete separation results in this time; running longer has little effect on the results. Results could be closely duplicated, at least to within 2%. This is more thoro sifting than is practiced in milling, but it is better for testing.

After the granulation test was made on each sample, the flour was saved from each sieve, and one sample was made of all the flour that collected on the No. 10 sieve. The same was done with all that collected on the Nos. 11, 12, 13, 14, 15, and 16 sieves, and also the part that went through the No. 16 sieve. When completed we had 8 samples of flour, each of a different granulation. A baking test was then made on each sample. To make the test still better, 40 samples of flour were used in the granulation test from which the 8 samples were collected.

The baking test was made as follows:

340 grams flour
3% sugar
2% salt
2% shortening
2% yeast
Absorption, 60 to 62
Temperature of dough, 80° F.
Temperature of dough during fermentation, 80° F.
Time of first fermentation, 120 min.
Time of second fermentation, 60 min.
Time of third fermentation, 40 min.
Time of fourth fermentation, 20 min. (on the bench)
Time of proof, 60 to 75 min. (until ready)

All doughs were mixed 5 minutes in a Bachman mixer, molded by machine, and baked for approximately 30 minutes at 400° F.

Table I shows the granulation test for each of the 40 samples, with the corresponding analysis and baking test. This table requires careful study. A few of the most important conclusions to be drawn will be mentioned.

Discussion of Table I

1. Only a small portion of all flour we tested was too granular to pass through a No. 10 or a No. 11 cloth. The small amount found on these was probably due to leakage of sifters and not intentional. There was so little of this coarse material that even after sifting 40 samples we did not have enough for a baking test.

2. From 40% to 60% of all flours passed through a No. 16XX, because our experimental sifting was longer than is usual in the flour mill. It is not because the cloth was bolted bare, as from 10% to 15% of the flour remained on top of this sieve.

TABLE I

GRANULATION TESTS — MONTANA FLOURS

Sample No.	Percentage of Separation: On 10	On 11	On 12	On 13	On 14	On 15	On 16	Through 16	Analysis and Baking Tests: Crude Protein	Ash	Loaf Volume	Grain	Texture	Color	Total
									%	%	cc	score	score	score	
565	0	0	2	2	9	8	13	66	11.3	0.43	2300	100	100	95	295
632	0	0	3	5	13	11	12	56	11.5	.41	2320	95	100	105	300
705	½	0	7½	6	12	10½	10½	53	10.8	.43	2350	95	100	95	290
609	0	0	5	7	13	10	11½	54	10.4	.38	2290	100	95	105	300
627	1	0	8	6	11	8	12	55½	10.8	.42	2280	100	96	93	290
631	0	½	9	6	14	11½	12	43	11.3	.47	2300	96	100	105	301
604	1	2	9	7	11	11	10	50	11.9	.46	2260	100	100	98	298
708	½	0	5	5	11	9	10	60	12.4	.46	2320	96	100	96	292
564	½	1	9	7	13	10	12	44	12.3	.51	2340	95	95	90	280
709	0	1	2	3	9	7	15	64	12.1	.42	2340	100	95	95	290
563	0	1	8	8	12	11	13	46	10.9	.73	2320	95	95	90	280
751	0	0	1	2	9	10	13	64	13.5	.44	2350	93	100	95	290
750	0	0	5¼	4	9½	9	14	58½	11.5	.36	2300	95	95	96	286
476	1½	½	8	6	13	8	11	50	11.7	.46	2350	102	102	102	306
479	1	0	8	8	16	15	16	37	11.2	.42	2240	96	98	95	289
477	½	0	4	2	11	10	12	60	12.6	.48	2340	102	100	95	297
480	1	0	5	7	17	35	17	18	10.6	.55	2200	95	95	95	285
504	0	1	9	11	16	14	13	33	11.9	.47	2290	100	100	95	295
497	½	0	7	6	10	8	10	59	11	.49	2310	95	95	105	295
531	½	0	3	7	11	10	10	59	11.4	.46	2300	100	100	96	296
530	½	0	8	8	12	9	7	54	11.3	.41	2300	97	97	102	296
529	½	0	4	5	10	8	12	60	12.5	.44	2350	95	95	95	285
494	½	0	8	9	11	8	11	51	12.3	.54	2350	98	100	105	303
707	½	0	6	7	11	10	14	52	12	.39	2350	100	100	102	302
706	½	0	6	3	11	9	11	58	12.3	.43	2340	100	99	95	294
419	0	½	9	11	17	15	32	15	11.3	.37	2300	100	100	96	296

Granulation, chemical and baking tests made on 44 different samples of Montana Flours, season of 1927-1928.

TABLE I *Continued*

GRANULATION TESTS—MONTANA FLOURS

Sample No.	On 10	On 11	On 12	On 13	On 14	On 15	On 16	Through 16	Crude Protein	Ash	Loaf Volume	Grain	Texture	Color	Total
	Percentage of Separation								Analysis and Baking Tests						
430	0	½	8	8	14	11	13	45	13	.45	2400	102	103	103	308
400	½	1	4	7	14	12	15	44	11.5	.36	2350	100	96	96	292
432	1½	3	12	10	15	12	12	35	11.1	.41	2370	100	101	99	300
478	1	2	11	7	14	14	12	40	11.4	.47	2250	102	102	95	299
452	1	1	11	7	11	36	6	29	11	.51	2280	98	98	100	296
503	0	0	5	4	12	10	10	58½	11.1	.41	2300	102	103	105	311
882	½	0	5	5	8	10	11	59	11.6	.40	2350	100	100	100	300
710	½	0	7	6	13	10	12	51	11.5	.38	2350	101	101	102	304
354	½	0	5	6½	11	11	15	52	11.6	.40	2350	100	100	100	300
	1	0	7	8	13	13	15	43	11.5	.40	2350	100	100	100	300
	½	0	6	6	13	12	14	48	11.5	.38	2350	101	101	102	304
	½	0	4	3	11	8	13	60	11.5	.38	2350	101	101	102	304
	0	0	3½	5	11	10	11	60	11.7	.45	2300	98	98	97	203
	0.2	0.5	6	6	10	8	16	53							
	.1	.6	6	5	10	9	17	53							
	.2	.5	6	5	10	9	17	51	11.6	0.40	2350	100	100	100	300
	.2	.7	7	6	10	9	14	51							
	0.2	0.7	7	6	9	8	14	54							

Granulation, chemical and baking tests made on 44 different samples of Montana Flours, season of 1927-1928.

3. Altho the finest flour cloth customarily used in a mill is No. 12XX, it is evident that most of the flour is finer than necessary to pass through this size.

4. The commonly accepted judgment as to the granulation of the flour (that is, by feeling it) is determined by the portion that will not pass through a No. 16XX cloth.

5. The 10 flours of finest granulation graded: Volume, 2320 cc. The total bread score was 295.2, made up of color 97.2, texture 99, and grain 99.

6. The 10 flours of coarsest granulation graded: Volume, 2306 cc. The total bread score was 294.9, made up of color 97.2, texture 100, and grain 97.7. It will be noted that the 10 flours of finest granulation had a little better texture but the grain was not quite so even.

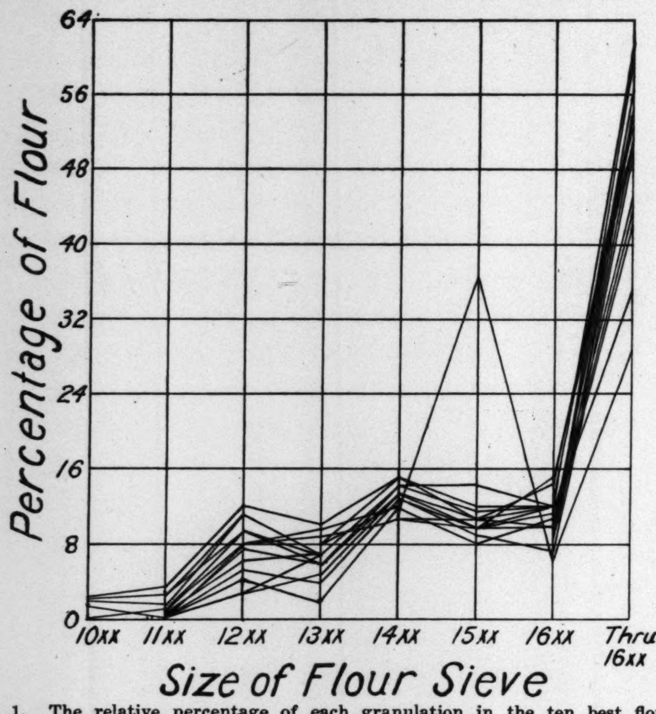

Figure 1. The relative percentage of each granulation in the ten best flours tested, with the bread score 296 or over.

7. Of the flour of highest bread score (No. 503), 58½% would pass through a No. 16XX cloth, nothing on a No. 10XX; nothing on a No. 11XX, 5% on a No. 12XX, and 4% on a No. 13XX. This flour was very fine and contained little coarse material.

8. Of the flour of lowest bread score (No. 564), 44% would pass through a No. 16XX cloth and much of it was coarse, ½% on a No.

10XX, 1% on a No. 11XX, 9% on a No. 12XX, 7% on a No. 13XX, and 13% on a No. 14XX.

Still further to connect the granulation with the quality of bread that could be made from it, we selected the 10 flours of best bread score and plotted a curve (Fig. 1), using as ordinates the percentage of flour found on each stream after making the granulation test, and as abscissae the streams from No. 10 to No. 16, and a last division of the part that went through the No. 16XX.

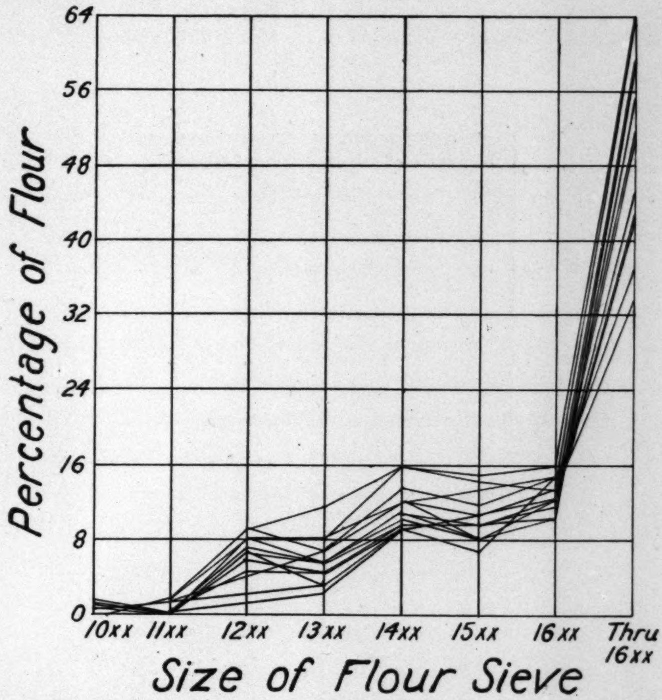

Figure 2. The relative percentage of each granulation in the ten poorest flours tested, with the bread score 295 or less.

Figure 2, plotted in the same way, represents the 10 flours of lowest bread score. All these were very good flours; hence we would not expect to find great differences. The most significant features are on the No. 11XX, 12XX, 13XX, and 14XX lines. The 10 best flours showed a more gradual rise in the percentage of fineness, that is, in the best flours the granulation showed the most even increment of fine flour. In other words, the flour went through all the steps from coarse to fine and contained a little of each granulation, the largest percentage being fine, the next largest a little coarser, and on down to a very small percentage of fairly coarse flour such as would be caught on No. 11XX and 12XX cloths.

TABLE II

TABULATIONS OF CHEMICAL AND BAKING TESTS OF FLOUR OF EACH GRANULATION

Test	Test and bakings on each granulation					
	On 12-13 photo 11	On 14 photo 12	On 15 photo 13	On 16 photo 14	Through 16 photo 15	Standard photo 7
Ash %	0.41	0.39	0.42	0.41	0.48	0.39
Moisture %	12.8	12.8	12.8	13.0	13.0	13.5
Flour color, hue	*	†	†	†	†	*
Crude protein %	10.7	11.22	11.7	12.08	11.5	11.8
Wet gluten %	29.5	31.4	31.8	32.8	32.3	32.0
Gluten color, hue	*	‡	§	§	§	*
Gluten quality	§ ‖	§ ‖	‖	‖	‖	‖
Absorption %	60.0	61.0	61.0	61.0	61.0	60.0
Viscosity	130	137	143	149	114	125
Fermentation time, hr. & min.	4:18	4:20	4:8	4:20	4:18	4:20
Loaves per bbl.	296	298	298	298	298	298
Loaf volume, cc.	2250	2300	2300	2320	2290	2350
Bread color, score	90	100	99	100	96	100
Bread texture, score	100	100	100	100	102	100
Bread grain, score	102	100	100	100	102	100

*Yellowish, †slightly gray, ‡good, §very good, ‖tough.

Discussion of Table II

Baking and Chemical Analysis on Each Granulation

See loaf 11 Flour on Nos. 12XX and 13XX
See loaf 12 Flour on No. 14XX
See loaf 13 Flour on No. 15XX
See loaf 14 Flour on No. 16XX
See loaf 15 Flour through No. 16XX

Ash.—The highest ash was found in the flour going through No. 16XX cloth. This probably comes from the break flour. The break flour is naturally subjected to more shattering as it is ground than the other flour, and this produces a large amount of fine dust.

Protein.—The highest protein was contained in the flour collected on No. 16XX, probably because the fine dust of the break flour has passed through the No. 16XX; the best fine middlings flour remaining on the No. 16XX.

Absorption.—The absorption was practically the same for all except a portion on Nos. 12XX and 13XX, which was 1% lower than the others.

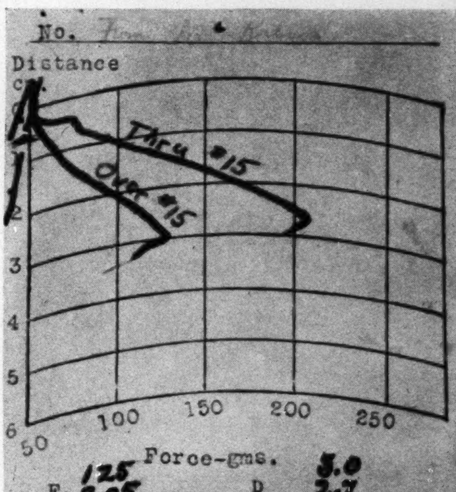

Figure 3. Specimen record of a test of the quality of gluten in the coarse and fine fraction of a typical flour.

Gluten quality.—The best gluten was in the flour that collected on the No. 15XX and No. 16XX cloths. To test the gluten quality we use a machine that registers the pressure required to stretch the wet gluten, and also the distance any given pressure will stretch the gluten to the breaking point. This machine is described in detail by Mr. James (see Cereal Chemistry vol. 4, No. 6). The gluten is washed from a sample of flour and left in the water until thoroly hydrated.

It is then placed in the machine and a record is made of the force required to break the gluten film and the distance that the film can be stretched by all pressures up to the breaking point.

Figure 3 represents the result of a test made on the portion from these samples going through a No. 15XX and a portion that was too granular to go through a No. 15XX. The upper curve represents the fine flour and the lower curve the coarse flour. The interpretation of these curves is that it took a force of 125 grains to break the gluten of the granular flour and of 205 grains to break that of the fine flour. The distance that the gluten from the granular flour could be stretched before baking, however, was 3 cm. compared to 2.7 cm. for the fine flour. That is, the gluten of the fine flour was tougher than that of the granular flour, but could not be stretched so far without breaking. The fine flour is tougher, but the granular flour is more elastic. Tough gluten is characteristic of this wheat. After the gluten has passed through fermentation it loses its toughness and gains elasticity. The granular flour, having more elasticity to start with, has a valuable characteristic. This is probably responsible for the slight tendency of a moderately granular flour to have an even grain.

Volume.—The best volume was obtained from the flour that collected on the No. 16XX; the next from that on the No. 14XX, the next on the No. 15XX, and least on the No. 12XX and No. 13XX.

Color.—The flour that collected on Nos. 14XX, 15XX, and 16XX produced bread with the best color score. The granular flour on Nos. 12XX and 13XX had a low color score. The fine flour that passed through the No. 16XX had a higher ash content and this brought down the color score; hence we are unable to say what the color would have been if the ash had been as low as in the other separations.

Texture.—The part passing through No. 16XX produced bread of the best texture. The other separations were alike.

Grain.—The flour remaining on the No. 13XX had the most even grain; that passing through the No. 16XX was very even. All through this experiment we were impressed by the fact that even grain seems to depend upon evenly distributed granulation, by which we mean that a flour containing a small amount of coarse granulation, such as that separated by No. 12XX; a little more of a little finer material such as that separated by No. 13XX; still more of finer material such as that separated by No. 14XX, and so on, with a gradually increasing fineness.

General Conclusions

The granulation of a flour makes only a little difference in its baking quality; the big difference is due to the wheat from which it is made. A flour of medium granulation has a tendency to produce a more even grain. Considering volume, color, texture, and grain equally. the best flour is found in the 30% to 40% of medium granulation, That is, if we sift out the coarsest and the finest flour until we have about 40% left, of medium granulation, we have the best baking quality.

Even granulation.—The more even the granulation the better the baking quality (see loaves 7, 8, 9, and 10).

TABLE III

A 95% Patent Flour Before and After Regrinding to Finer Granulation

Granulation test	Standard patent reground to finer granulation photo 10	Standard patent before grinding, standard granulation photo 9
On No. 10	0	½
On No. 11	0	0
On No. 12	½	4
On No. 13	1½	5
On No. 14	4½	10
On No. 15	4½	10
On No. 16	9	10
Through No. 16	80	60½
	Analysis and Baking Test	
Ash	0.41	0.41
Flour color	*	*
Protein	11.8	12
Wet gluten	31.5	32.8
Gluten color	†	†
Gluten quality	‡	‡
Absorption	61	61
Total expansion	4:20	4:20
Loaves per barrel	298	298
Volume	2320	2320
Color	100	100
Texture	100	100
Grain	103	100

*Yellow, †slightly yellow, ‡tough.

Discussion of Table III

Table III shows a 95% patent flour from which the coarser portion was sifted, reground on a small mill, and resifted; the grinding and sifting being continued until 20% more passed through a No. 16XX than at the beginning. The improvement in grain is noticeable.

TABLE IV

AN 85% PATENT FLOUR BEFORE AND AFTER REGRINDING TO FINER GRANULATION

Granulation test	Patent reground to finer granulation photo 8	Patent before grinding standard granulation photo 7
On No. 10	0	½
On No. 11	0	0
On No. 12	2	6
On No. 13	3	5
On No. 14	8	10½
On No. 15	9	10
On No. 16	12	12
Through No. 16	66	56
	Analysis and Baking Test	
Ash	0.38	0.38
Flour color	*	*
Protein	11.8	11.5
Wet gluten	31.8	31.3
Gluten color	†	†
Gluten quality	‡	‡
Absorption	61	61
Total expansion	4:30	4:20
Loaves per barrel	298	298
Volume	2310	2320
Color	100	100
Texture	103	100
Grain	100	100

*Yellowish, †slightly yellowish, ‡tough.

Discussion of Table IV

Table IV shows similar treatment on 85% patent flour from the same wheat. This showed a considerable improvement in texture, altho from the nature of the flour less improvement would be expected than from a 95% patent.

Fermentation

The statement is often made that one of the reasons for uniform granulation is that fine flour ferments faster than coarse flour; hence a mixture of the two will cause uneven fermentation. When mixed in the same dough it is said that a fine flour will be over-fermented and a coarse flour under-fermented. To test this we separated the fine flour from the coarse by sifting on a No. 15XX cloth until approximately 50% went through. We use for this purpose an 85% patent from Montana Marquis.

Figure 4 illustrates the result of this test. The test was made as follows:

200 grams flour	7 grams salt
500 cc. distilled water	7 grams sugar
7 grams yeast	Temperature, 80° F.

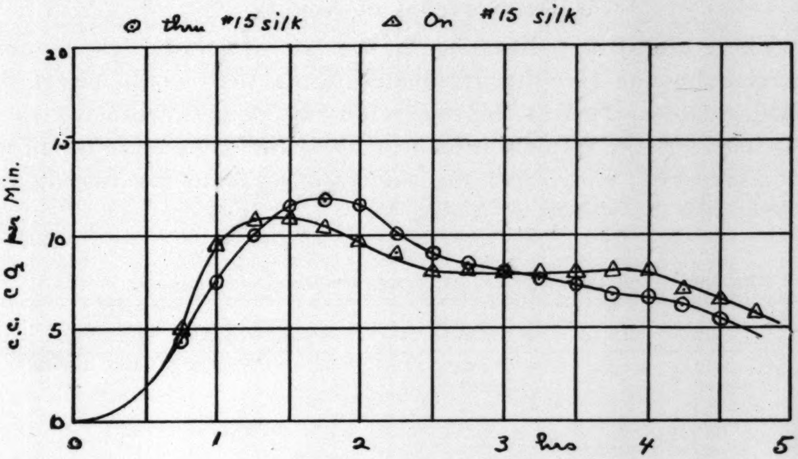

Fig. 4. Rate of Fermentation of Fine and Granulated Flour

The rate at which carbon dioxid was given off was measured by the rate of flow of distilled water. The apparatus is so designed that it automatically draws a curve on a revolving drum on which a sheet of cross-section paper is fastened. The fermentation progressed uniformly for 45 minutes in both the granular and the fine flour. At the end of 45 minutes the granular flour fermented a little faster than the fine flour. At the end of 75 minutes the granular flour started to flow down and the fermentation of the fine flour became equal to that of the granular flour. From 80 to 180 minutes the two were equal, and from 180 to 300 minutes the fermentation of granular flour was slightly faster. Considering the whole fermentation period (4 hours), the rate was slightly in favor of the granular flour. It is not true that the fine flour ferments faster than the coarse, but both ferment at nearly the same rate.

Conclusions

1. The granular flour produced a little more acid than the fine flour during the first, second, and third hours of fermentation.

2. At the end of four hours the dough made from granular flour had the same acidity as that made from fine flour.

3. It is evident, from the rate of evolution of gas, from the rate of increase in acidity, and from the volume of the dough during fermentation, that both fine and medium granular flour such as is ordinarily made in good milling had practically the same rate of fermentation.

Development of Acidity

There might be a difference in the rate of acidity development between tne fine and the granulated floors that would affect the baking quality. To test this we made two doughs from the same flour used for the fermentation test, used the same formula as for the baking test, and tested the acidity every hour for four hours, with results as shown in Table V.

TABLE V

Development of Acidity in Dough Made from Fine and Granular Flours

	Granular flour over No. 15XX	Fine flour through No. 15XX
	pH	pH
Flour	5.80	5.93
Dough	5.70	5.86
Dough after 60 min.	5.78	5.88
Dough after 120 min.	5.71	5.73
Dough after 180 min.	5.54	5.66
Dough after 240 min.	5.46	5.46

We also measured the volume of the same doughs during fermentation. The results are shown in Table VI.

TABLE VI

Volume of Dough Made from Fine and Granular Flours

	Granular flour over No. 15XX	Fine flour through No. 15XX
	cc.	cc.
After 60 min.	750	700
After 120 min.	1300	1300
After 180 min.	1800	1850
After 240 min.	1800	1800

LABOR SAVING DEVICES FOR THE COMMERCIAL PROTEIN TESTING LABORATORY[1]

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(Received for publication January 21, 1929)

Labor saving devices are always of interest. The equipment briefly described has been used for several months in the Montana Grain Inspection Laboratory and has been found to contribute to both the speed and the accuracy of making protein tests. It is simple in construction and comparatively inexpensive.

1. Carriages for Holding Kjeldahl Flasks

These are of two kinds and are used for different purposes. (A) To hold flasks upright. This carriage consists of a frame 20 x 32 inches mounted on legs 30 inches long. Over the top of the frame is tightly drawn wire netting having meshes approximately 2½ inches square. About 11 inches under this a second netting is placed. A water tight pan 2 inches deep is securely fastened about 11 inches below the second netting. The carriage is mounted on casters and holds the flasks upright while they are being loaded preparatory to digestion. It also serves as a draining and drying rack after the flasks have been washed following the test. The rack has a capacity of 24 flasks while loading; twice that number while drying. Three such carriages are used in the Montana laboratory.

(B) Cooling rack. A low carriage with space for 24 flasks serves as a holder and cooling rack following digestion and while the flasks are being prepared for distillation. As soon as digestion is complete the flasks are stoppered with large cork stoppers and placed on the rack. This serves the two-fold purpose of hastening the cooling of the flasks and making the heaters available for other tests. This carriage is 24 inches high, 20 inches wide, and 65 inches long, and is mounted on casters. Two rows of holes are cut in the top near the edges. These holes are 3 inches in diameter and 2 inches apart. Midway between these rows of holes and 6 inches above them is fastened a notched board in such a way as to receive the necks of the Kjeldahl flasks when they are resting in the holes. This arrangement holds the flasks

[1]Approved by Director.

at an angle of 45 degrees and makes them readily accessible from both sides of the carriage.

2. Caustic Soda Burette

A rapid method of measuring the caustic soda solution without exposing it to the air for a long time is essential in protein testing. This is accomplished by using a glass tube 40 inches long with an inside diameter of 6.5 cm. and having a rubber stopper fitted in each end. This tube is fastened in an upright position near a shelf on which is placed a 10-gallon carboy of the solution. The carboy is fitted with a large funnel to provide a method of refilling it without removing it from the shelf. The solution is syphoned from the carboy to the tube through a hole in the stopper in the lower end of the tube. A second opening in this stopper, together with a small glass tube, rubber tube, and pinch cock, forms the opening by which the solution is drawn off. The tube is marked off into spaces 1.7 cm. in length, each of which equals approximately 60 cc. of the solution.

The upper end of the long tube extends above the top of the bottle. This insures against the solution overflowing and allows the opening of the syphon to fill the tube without attention. The rubber stopper that closes the upper end of the tube has a glass U tube fitted into it. This serves the double purpose of furnishing a limited air outlet and a place to attach a rubber hose to which suction is applied to start the syphon.

3. Aids in Filling Receiving Flasks

Every chemist in a commercial protein laboratory recognizes the necessity of a rapid, accurate method of filling the receiving flasks. The following simple devices have proved most satisfactory.

A. Acid burette—A short pipette of 20 cc. capacity is converted into an automatic burette by heating the tip in a flame and bending it to nearly a right angle. About half the stem is then cut off and a T-shaped glass tube with rubber connections, glass tip, and two pinch cocks is attached. The burette can be readily standardized by comparing it with one or more standard burettes by means of acid and alkali solutions of the same normality. If the direct method of reading the percentage of protein on the alkali burette is employed, the blank can be taken care of by making a mark the desired distance (0.2 or 0.3 cc.) below the 20 cc. mark.

With this type of burette it is possible to measure acid to the nearest drop, as the reading is made in the small stem where one drop

makes a difference of about 4 mm. in the level of the liquid. A careful comparison of this burette with the automatic burette having the two-way stop-cock that is commonly used for measuring acid into receiving flasks, shows that the former has the following advantages:

a. Greater accuracy.
b. Greater speed.
c. It is more convenient to read, as the surface of the liquid being read may be adjusted level with the eye of the operator.

B. Burette for distilled water.—A convenient method of adding distilled water to the receiving flask is effected by fastening a glass tube 40 inches long and having an inside diameter of about 2 cm. in an upright position at the edge of the shelf near where the acid is measured. The bottom is stoppered with a No. 3 rubber stopper having two holes. The tube is attached through one of these holes by means of an open syphon to a 5-gallon carboy of water on the shelf. A pinch cock and a large tip are attached to a short tube in the other hole and serve as an outlet through which the water is drawn.

The upper end of the tube extends to the top of the carboy. This insures against overflowing of the water when the syphon is left open, which is as soon as the carboy is in place. The desired amount of water can be easily judged by noting the distance the water level falls in the tube.

C. Tube for indicator.—A tube about 24 inches long and of the same size as that mentioned above serves as a convenient container for the indicator solution. The tube is attached to the edge of the shelf near the distilled water burette. The desired number of drops of solution is obtained by means of a special dropper attached to the end of a glass tube passed through the rubber stopper which closes the lower end of the tube.

This special dropper consists of a small rubber tube about 2 inches long, an ordinary glass tip such as is used for a burette, and a glass bead 5 or 6 mm. in diameter. The bead placed in the rubber tube serves as a valve, which opens and allows the solution to pass as pressure is applied with the thumb and finger to the surface of the tube over the bead.

This form of dropper is well adapted to use on an ordinary burette used in titrating. The amount of solution that passes can be controlled by the degree of pressure applied.

CAROTINOID PIGMENTS OF FLOUR

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(Received for publication September 1, 1928)

Color as a Function of Flour Quality

The color of wheat flour began to assume its greatest importance as a commercial factor shortly after the modern roller milling process was introduced. According to Dedrick (1924) the transition from the old style of milling with millstones to milling with metal rolls began in America about 1876, altho the roller type of mill was used in Budapest as early as 1844. At the same time purification of the middlings was accomplished to a greater degree than ever before by the introduction of the middlings "purifier." The history of the milling process and a description of the machinery employed is adequately covered in texts on milling, notably those by Dedrick (1924), Kozmin (1917) and Amos (1912).

It suffices for the present purpose to point out that the improvements referred to made it possible to produce much whiter flour, and there resulted a demand from the public for the whitest possible product. An outgrowth of this demand has been the introduction of chemical bleaching agents, which had their commercial beginning in patents granted to Frichot in 1898. The public demand for white flour has never diminished, and as a result chemical bleaching of one type or another is generally practiced in the United States. So far as we know, flour color of itself has no direct relation to flour strength as measured in terms of the colloidal properties of dough and enzyme phenomena. Curiously enough, it is regarded as a factor whose importance is scarcely second to other considerations more fundamental in their relation to the potentialities of the flour in baking. In the development of new wheat varieties, a strain having excellent characteristics except color is discriminated against or even discarded.

Factors Determining the Color of Flour

The visual appearance of dry flour is influenced by a variety of factors acting simultaneously. The resulting sensation is usually expressed by the single term color. Flour color is frequently defined as white, yellow, gray, dull, etc. Such terms lack definiteness. The

very inadequacy of descriptive words necessitates the careful use of terms employed by the physicist in defining color. The report of the committee on colorimetry of the Optical Society of America (1922) and the spectrophotometry report of the same society (1925) contain definitions that fully cover their respective fields. These two reports are the sources of the definitions and nomenclature used here.

The ordinary stimulus of color is radiant energy of certain wave lengths. The color sensation that results from such stimulation of the eye mechanism is specific in normal individuals for the wave length or frequency of the exciting stimulus. In order to perceive color and become aware of the color sensation it is necessary for the stimulus to travel from the source to the eye. The process by which radiant energy is thus propagated through space is called radiation; and it is apparent that radiant energy and radiation are not synonymous. Light is a form of radiant energy, and is defined as radiant energy multiplied by the visibility of the radiant energy. "Light" is a term reserved for the stimulus of color and is not synonymous with color that is the sensation produced when light affects the optic nerve. It should be emphasized that color is subjective and light objective. Such a commonly used phrase as "ultra-violet light" is a misnomer. Light may be considered to travel through space in the form of vibrations or wave motions. When white light is decomposed into its component rays the familiar spectrum results, the most conspicuous colors of which are usually termed violet, blue, green, yellow, orange, and red.

The phenomenon of light absorption is responsible for the observed color of bodies. If light traverses a body it is transparent. If all the wave lengths of light are transmitted in the same proportion in which they exist in white light, the body is colorless. If some of the light is absorbed in transmission, the body is transparent and colored. A solution is yellow because the yellow components of white light are transmitted and the others are absorbed. In the same way a body may be colored because of reflected light. If a body reflects all the wave lengths of white light in the proportion in which the light impinges upon it, it is opaque and white. If none of the impinging light is reflected or transmitted, the body is black. A fresh, clean surface of magnesium carbonate is white because it reflects the white light that impinges upon it with little absorption. Another object is green because it reflects a preponderance of green light rays, absorbing the others. The point to be emphasized is that *the color observed results always from the constituents of the impinging light that are not absorbed.*

The factors involved in the visual appearance of flour are (1) granulation, (2) moisture, (3) bran pigmentation, (4) foreign matter, and (5) natural carotinoid pigmentation. (Bailey, 1925, p. 194).

The granulation of flour has such a profound effect upon the dry material that it becomes a most important factor. When the granulation is coarse, the light source throws shadows that result in a gray, and the flour has less apparent brightness. The term employed by the physicist for this color attribute is brilliance. If a coarsely divided sample of flour is reduced to a finer state of subdivision, the flour will have a much whiter appearance, more free from gray, and its brilliance will be greater; it will reflect a greater percentage of the light that falls upon it. The refraction of light also plays a part in the visual appearance of flour, as there is a difference between the refraction of light in the dense endosperm particles and in the air spaces surrounding them. The coarser the granulation the larger is the effect.

The familiar Pekar test illustrates the influence of moisture on color. In the application of this test lower grades of flour become darker and the bran specks are more easily discerned. This darkening in color is due to enzymes, which originate in the outer layers of the wheat, and according to Bertrand and Mutermilch (1907) these are glutenase and tyrosinase. The test is chiefly of value to the miller in checking the efficiency of the bolting operation, and in making immediate, rough comparisons.

Bran pigmentation becomes a factor influencing flour color, as minute bran particles may contaminate the flour. This factor is possibly most important in flour milled from "red" wheats.

Much foreign matter as dirt, soil, and smut spores, may become a factor influencing color, depending upon the extent of the contamination and the efficiency of the grain-cleaning process that precedes grinding.

The carotinoid pigment content determines the yellowness of flour and affects that attribute of color called hue. Flours differ considerably in the amount of pigmentation they contain. Monier-Williams (1912) compared the absorption spectra of petroleum ether extracts of flour with similar solutions of pure carotin and came to the conclusion that the pigments were identical, or at least had no noticeably different absorption spectra.

Carotin is the natural pigment of the endosperm and inevitably makes its appearance in the flour. The other factors influencing color are fairly well under the control of the miller. Proper milling meth-

ods remove much of the color of refined grades of flour that is due to foreign matter and bran pigmentation. The natural carotinoid pigment content is the only factor that cleaning and mechanical milling does not influence. Of all the factors involved in the visual appearance of flour, chemical bleaching processes affect carotinoid pigment content alone, and it thus becomes the only factor that lends itself to positive, chemical control by the miller. Color measurements on flour extracts, using the ordinary fat solvents, deal with carotinoid pigmentation. It is conceded that other factors may be predominant in specific cases, but they are not concerned with the flour color measurements considered here.

Color analysis and measurement can be carried out in several ways. Shepard (1905) evolved a method for examining the color of dry flour in which he used Lovibond slides as a standard. Later, Jago (1911) used a Lovibond tintometer for the same purpose. In colorimetry, color is specified by reference to some arbitrary standard or stimulus that is found to produce the same color sensation, using one of the several types of colorimeters. The difficulty with colorimetric methods is frequently that the stimulus used as the standard is not identical with the stimulus of the color to be matched. Standards of several varieties have been used. Willstätter and Stoll[1] (1913, 1928), working with pure carotin, employed a 0.25 per cent solution of alizarin in chloroform, or a 0.2 per cent aqueous solution of potassium dichromate. The use of pure carotin as a standard is not practical. Schertz (1923), working with pure carotin solutions, used Lovibond slides in a Duboscq colorimeter, and demonstrated that they were not adequate for precise work. In the gasoline color value of flour, devised by Winton (1911), an 0.005 per cent aqueous solution of potassium chromate is the standard. That the hue of the reference solutions proposed is not satisfactory is manifested in the efforts of later workers to substitute others.

Jørgenson (1927 a, b) pointed out that solutions of potassium chromate and potassium dichromate in water are hydrogen-ion indicators. He proposed, therefore, to dissolve these salts in standard buffer solutions. After trying each over a wide range of H-ion concentration, he recommended the use of potassium chromate prepared in the proportion of 1.00 cc. of 0.05 per cent potassium chromate to 0.5 cc. M/15 disodium hydrogen phosphate plus 9.5 cc. M/15 potassium dihydrogen phosphate. That this solution is likewise inadequate may be inferred from his own words " . . . in varying the buffer mixtures in which the chromate is dissolved," it is possible "to prepare solutions possessing many dif-

[1] References to Willstätter and Stoll (1928) cite the authorized English translation of their "Untersuchungen über Chlorophyll" (1913) by Schertz and Merz.

ferent defined red-yellow and yellow color tones." It appears that increased difficulty results from such a procedure, as the color values obtained with different standards are obviously incomparable. The use of a standard of comparison having the same spectral absorption properties as carotin would eliminate much of the difficulty.

Another modification of the standard employed in evaluating the gasoline color value of flour was employed by Kent-Jones (1927), who used 10 cc. of 0.5 per cent potassium chromate solution and 1.5 cc. of 10 per cent anhydrous cobalt nitrate solution made up to a volume of 100 cc. with distilled water.

Schertz (1923) ascertained the error of colorimetric estimations of pure carotin by using the same concentration of carotin solution in both sides of a Duboscq colorimeter. The results he reported varied 33 1/3 per cent. If pure carotin solutions are so difficult to match when both sides of a colorimeter contain the identical pure substance, how much more difficult the situation becomes when a standard of unsatisfactory hue is used!

In consequence of the difficulties referred to, it appears improbable that the colorimeter can be used as an instrument of precision for the measurement of the carotinoid pigment content of flour. For exact work a more fundamental basis of color specification is essential. For this reason the methods of spectrophotometry are employed. Spectrophotometry provides a method for completely specifying the stimulus of color independent of material color standards. The identical radiant energy that evokes the color is specified and its spectral distribution measured. These measurements are not only independent of color standards but are also independent of abnormalities of the observer's vision and other personal eccentricities that make colorimetric methods so difficult.

Definitions and Nomenclature of Spectrophotometry

"Spectrophotometry may be defined as the visual measurement of relative radiant energy as a function of wave length or frequency. This energy may be that emitted by incandescent or other light sources, or it may be that transmitted, absorbed, or reflected by transparent or absorbing materials." (Optical Society of America, Spectrophotometry Report—1925) Instruments for making such measurements are called spectrophotometers or spectroradiometers.

As the use of the spectrophotometer involves the use of terms not hitherto employed in the discussion of flour color measurements, these terms are defined here. The definitions are taken from the report of the committee of the Optical Society of America on colorimetry (1922)

and on spectrophotometry (1925). The spectrophotometer can be used for both reflection and transmission measurements, but as this discussion will be confined to transmission measurements exclusively, reflection terms will not be defined.

When light from a homogeneous source of radiant energy passes through a homogeneous, isotropic medium having parallel surfaces perpendicular to the direction of propagation, the following terms are employed:

E = the radiant energy of the source of light.
E_1 = radiant energy incident on the first surface.
E' = radiant energy reflected by the first surface.
E_{I} = radiant energy transmitted by the first surface.
E_2 = radiant energy incident on the second surface.
E'' = radiant energy reflected by the second surface.
E_{II} = radiant energy transmitted by the second surface.

In correcting for losses by reflection the above definitions permit the use of the expression $E_{\mathrm{I}} = E_1 - E'$.

Transmission (T) may be defined as the ratio of the radiant energy transmitted by the second surface to the radiant energy incident on the first surface, and is expressed:

$$T = \frac{E_{\mathrm{II}}}{E_1}$$

Transmittance (T) may be defined as the ratio of the radiant energy incident on the second surface to the radiant energy transmitted by the first surface, and is expressed:

$$\mathrm{T} = \frac{E_2}{E_{\mathrm{I}}}$$

Transmissivity (t) is equal to the "b" root of the transmittance, where "b" is the distance between the bounding surfaces. This relation, known as Lambert's law, is expressed:

$$t = \sqrt[\mathrm{b}]{\mathrm{T}}$$

The transmissive exponent (i) is expressed by the following relation:

$$i = -\log_e t$$

The transmissive index (k) is given by:

$$k = -\log_{10} t$$

In dealing with transparent solutions where T_{sol} = transmission of a given cell containing the solution, and T_{sov} = transmission of the same or a duplicate cell containing pure solvent, etc., the term transmittancy

is used. It is defined as the ratio of the transmission or transmittance of a cell containing the solution to the transmission or transmittance of a duplicate cell containing the solvent, and is expressed:

$$\mathbf{T} = \frac{T_{sol}}{T_{sov}} = \frac{\mathrm{T}_{sol}}{\mathrm{T}_{sov}}$$

In the discussion which follows it is the transmittancy that is actually measured by the spectrophotometer in making transmission measurements. A study of the above definitions will reveal that the transmittancy measured has been corrected for reflection of light at the surfaces, and for any light absorption by the solvent.

Specific transmissivity ($\mathbf{t}$) is expressed by the relation:

$$\mathbf{t} = \sqrt[bc]{\mathbf{T}}$$

where "c" is the concentration of the dissolved substance in centigrams per liter, and "b" is the thickness of the layer of solution in centimeters. Transmissivity and specific transmissivity differ in that the latter takes into consideration the concentration of the solution as well as the thickness. This relation is known as Beer's law, and it forms the basis of the method described for the quantitative estimation of carotin in solution.

The specific transmissive exponent ($\mathbf{i}$) is expressed by the relation:

$$\mathbf{i} = -\log_e \mathbf{t}$$

The specific transmissive index ($\mathbf{k}$), also called the "extinction coefficient" is expressed as follows:

$$\mathbf{k} = -\log_{10} \mathbf{t}$$

Since $\mathbf{t} = \sqrt[bc]{\mathbf{T}}$ it follows from the above expression that

$$\mathbf{k} = -\log_{10} \sqrt[bc]{\mathbf{T}} = -\frac{1}{bc} \log_{10} \mathbf{T} \text{ and } bc\,\mathbf{k} = -\log_{10} \mathbf{T}$$

If b and $\mathbf{k}$ are kept constant the concentration is proportional to the negative logarithm of the transmittancy. Beer's law does not always strictly hold, but Schertz has shown (1923) that for dilute solutions of carotin the relation holds very well. He determined the specific transmissive index for carotin in petroleum ether and alcohol solution to be 1.9148.

The source of radiant energy used in the measurement of carotin solutions is important. These solutions have the greatest absorption at the shorter wave lengths. Thus the transmittancy values for different concentrations of carotin at the red end of the spectrum are so great

that differences in concentration are not easily discerned. The absorption of light at the blue end of the spectrum is, however, very great, and appreciable differences are manifest when very small differences in concentration of carotin are employed. As the ordinary source of white light provided by the instrument used did not give radiant energy of sufficient intensity for use at the blue end of the spectrum, it was necessary to use another light source. This was easily furnished by a mercury vapor arc lamp that emits light with a bright band at a wave length of 435.8 mμ.

Description of the Spectrophotometer

The spectrophotometer used in these studies was a Keuffel and Esser color analyzer, which has been described by Keuffel (1925a, 1925b, 1925c). This instrument is designed for measuring the selective transmission or reflection of substances in the visible range of the spectrum. In addition to the light source furnished with the instrument we made provision for using a mercury vapor arc lamp in transmission measurements. The light was condensed by a suitable lens which was the only addition made to the optical system of the instrument.

The Operation of the Spectrophotometer

The first operation in using the spectrophotometer for the measurement of the transmittancy of carotin solutions was to turn on the mercury vapor arc lamp. It was necessary to wait until the lamp became hot, otherwise small mercury droplets obscured the photometric field. The intensity of the light increased when the lamp became hot. This was a distinct advantage. It was then necessary to adjust the position of the lamp so that the two halves of the photometric field were equally illuminated at the wave length 435.8 mμ. The instrument was ready for use when this condition was met. The exhaust fan used with the regular light source was not needed and was disconnected.

One cell was filled with the solvent, petroleum ether, and placed in the lower part of the sample holder, and a duplicate cell was filled with the pigmented solution and placed directly above it. The manner of preparing the solution is described elsewhere. The photometer was set in motion and the photometric fields were matched by adjusting the relative position of the rotating discs by turning the photometer scale. The speed of the motor driving the sectored discs was altered by means of a rheostat until flicker was eliminated. The per cent transmittancy was read directly. In all the determinations here recorded ten readings were taken in every case, and the average value was

TABLE I

TRANSMITTANCIES OF CAROTIN IN PETROLEUM ETHER SOLUTION USING THE MERCURY VAPOR ARC LINE 435.8 mμ
CALCULATED FROM THE DATA OF SCHERTZ.*

Concentration of pigment centigrams per liter	1 cm. cell			2 cm. cell			10 cm. cell		
	$-\log_{10}T$	T (Transmittancy)	Per cent transmittancy	$-\log_{10}T$	T (Transmittancy)	Per cent transmittancy	$-\log_{10}T$	T (Transmittancy)	Per cent transmittancy
0.01	0.019148	0.95686	95.7	0.038296	0.91559	91.6	0.191480	0.64345	64.3
0.02	0.038296	0.91557	91.6	0.076592	0.83831	83.8	0.382958	0.41403	41.4
0.03	0.057444	0.87611	87.6	0.114888	0.76756	76.8	0.574440	0.26642	26.6
0.04	0.076592	0.83831	83.8	0.153184	0.70278	70.3	0.765916	0.17142	17.1
0.05	0.095740	0.80216	80.2	0.191480	0.64346	64.3	0.957408	0.11030	11.0
0.06	0.114888	0.76756	76.8	0.229776	0.58916	58.9	1.148879	0.07097	7.1
0.07	0.134036	0.73445	73.4	0.268072	0.53942	53.9	1.340360	0.04567	4.6
0.08	0.153184	0.70277	70.3	0.306368	0.49389	49.4	1.531836	0.02939	2.9
0.09	0.172332	0.67246	67.2	0.344664	0.45220	45.2	1.723320	0.01892	1.9
0.10	0.191480	0.64346	64.3	0.382960	0.41403	41.4	1.914800	0.01217	1.2

*F. M. Schertz. J. Agr. Research 26:383 (1923).

reported. When the transmittancy was less than 25 per cent the sectors were lowered in the manner provided, and the reading was then divided by four.

The wave length scale, which is marked for the D line of the spectrum, was frequently calibrated using a sodium flame. Another adjustment requiring careful attention was the matching of the photometric field at 100 per cent transmittancy.

Quantitative Determination of Carotin

The spectrophotometric method for the quantitative estimation of carotin was first employed by Schertz (1923). He determined the transmittancies of solutions of pure carotin of known concentration. The values he obtained with a 2 cm. cell using petroleum ether as the solvent and the mercury vapor line 435.8 mμ., have been calculated for integral concentrations of carotin and are reported in Table I along with similar values for 1 cm. and 10 cm. cells, also calculated from his data. (The Keuffel and Esser Color Analyzer or spectrophotometer used in these studies comes equipped with only the 1 cm. and 10 cm. cells). These data are plotted in the following manner. The concentration of carotin is plotted on an equal division scale along the axis of abscissa. The percentage transmittancy is plotted on a logarithmic scale along the left-hand ordinate, while the right-hand ordinate indicates the corresponding values for the negative logarithm of the transmittancy. The accompanying graph (Fig. 1) shows the straight-line relation between the concentration of pigment and the negative logarithm of the transmittancy for each of the three cells. In the work reported later it was found that the 10 cm. cell was most convenient, and the coordinates were chosen of such dimensions as to permit the plotting of this line at an angle of approximately 45°.

The data represented by the graphs in Figure 1 can be used in the quantitative estimation of carotin.[2] In making use of the curve the transmittancy of the unknown petroleum ether solution of carotin is determined. The concentration of carotin corresponding to this transmittancy is found by locating the value for the per cent transmittancy on the left-hand ordinate, tracing this point horizontally until it intercepts the curve for the cell used, and projecting this point vertically along the ordinate until it intercepts the scale along the abscissa where the concentration of carotin is read.

The concentration of carotin may be calculated from the relation,

$$bc\mathbf{k} = -\log_{10}\mathbf{T}$$

2 Large copies of this chart can be had at cost by addressing the authors.

which has been discussed previously. In this equation the concentration is the only unknown quantity after the transmittancy has been determined.

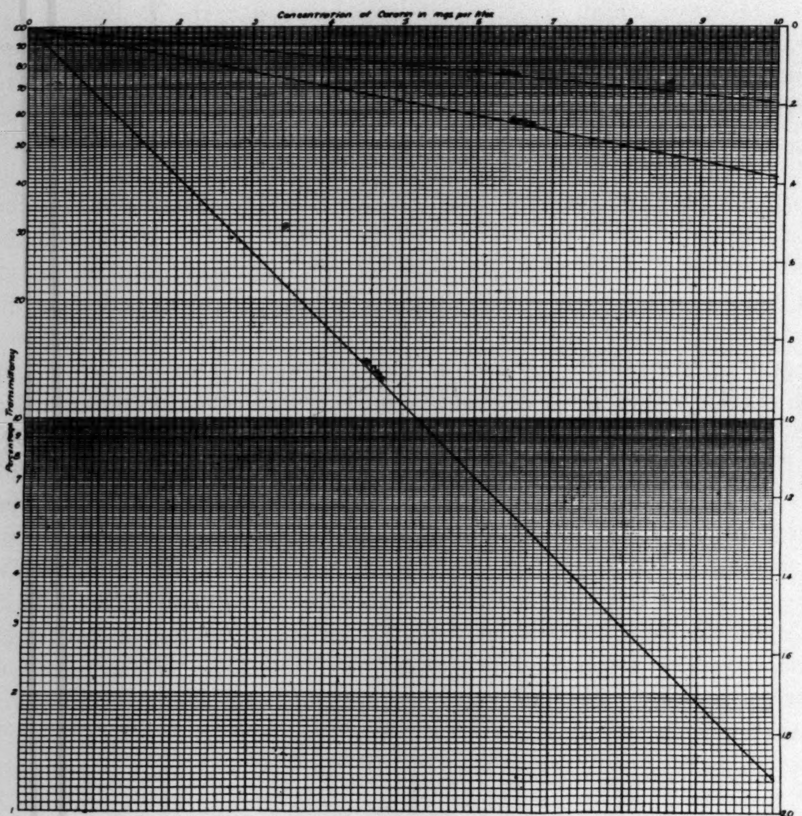

Fig. 1. Transmittancies of Petroleum Ether Solutions of Pure Carotin in 1 cm., 2 cm., and 10 cm. cells, Using the Mercury Vapor Line of 435.8mμ as the Light Source.

The values for the transmittancy at a given cell length may be converted into values of another cell length by means of the formula

$$T^{\frac{1}{t}} = T_1^{\frac{1}{t_1}}$$

where T and T_1 are transmittancies and t and t_1 cell length. A specially designed slide rule may be had for making this calculation. It must be borne in mind when making use of the data that the value for the specific transmissive index of carotin is different for different solvents. Petroleum ether and absolute ethyl alcohol give the same values, but other solvents investigated do not. It is also essential that the light source have the specified wave length of 435.8 mμ.

Determination of the Specific Transmissive Index (k) for Carotin in Absolute Alcohol or Petroleum Ether

The method developed later for the quantitative determination of flour color from spectrophotometric examination of the gasoline extracts of flour is based on the method by Schertz (1923) for pure carotin. The curves, Figure 1, show the relation between the per cent transmittancy and the concentration of carotin in petroleum ether solution calculated from Schertz's data. It appeared desirable to determine again the specific transmissive index of carotin in petroleum ether solution in order to confirm the value reported by Schertz, and to afford further proof of the quantitative relation between carotin concentration and per cent transmittancy.

TABLE II

SPECIFIC TRANSMISSIVE INDEX (k) FOR CAROTIN IN PETROLEUM ETHER OR ABSOLUTE ALCOHOL, DETERMINED IN A 10 CM. CELL AT A WAVE LENGTH OF 435.8 $m\mu$

Carotin concentration centigrams per liter	Per cent transmittancy	$-\log_{10}T$	k
0.0204	40.6	0.39147	1.918
0.0340	22.3	0.65170	1.916
0.0476	12.28	0.91080	1.913
0.0632	6.13	1.21289	1.919
		Average..........................	1.9165

Pure carotin, prepared as described elsewhere, was used in this determination; 0.0017 grams was dissolved in a liter of absolute alcohol that contained about 300 cc. of petroleum ether to facilitate solution. Dilutions of this solution were made and the transmittancies measured spectrophotometrically in a 10 cm. cell using the mercury vapor line 435.8 $m\mu$.

The data secured are recorded in Table II. The average value found for the specific transmissive index was 1.9165, which is practically identical with the average value, 1.9148, reported by Schertz. The close agreement in these results also indicates that the sample of carotin used for these determinations and subsequent comparisons was pure.

Comparison of the Absorption Spectra of Pure Carotin Solutions with Extracts of Flour and Bran

Introduction.—The determination of flour color, by means of spectrophotometric measurements of the transmittancies of gasoline extracts, is based on the assumption that the pigment of flour is essentially carotin. A comparison of the absorption spectra of carotin in petroleum ether solution with the absorption spectra of petroleum ether

extracts of flour was made by Monier-Williams (1912). From the results of his measurements he came to the conclusion that the coloring matter of flour was either carotin or a substance or mixture of substances so closely allied to carotin that their absorption spectra were practically identical. It was thought advisable to check the results of these findings, and in order to have carotin for a basis of comparison, pure carotin was isolated from carrots. A variety of methods has been used for this purpose as well as several sources. A convenient summary of these is given by Palmer (1922).

Schertz (1925a) prepared carotin from carrots and his method, with slight modifications, was followed here.

Isolation of pure carotin.—One hundred pounds (2 bushels) of carrots were scrubbed carefully with a brush. They were then cut in slices about 2 mm. thick using a rotary hand slicer. The sliced carrots were partially dried in hot-air heated drawers. The temperature of these drawers was variable, but it was attempted to keep the temperature below 50°C. The drying required approximately 24 hours. The dried carrots were ground in a Weber mill using a fine-mesh sieve. The ground carrots were then dried in a large vacuum pan drier at 50°C. for 4 hours. The weight of the dry ground material obtained was 3432 grams.

The ground, dried carrots were placed in a large glass funnel, the stem of which was covered or plugged with a layer of cotton. Petroleum ether (B.P. 30-60°C.) was added and the bottom outlet of the funnel was stopped. Petroleum ether and the ground carrots were allowed to stand in contact over night. Afterward the stopper in the bottom was removed and the petroleum ether percolated through slowly. Fresh additions of petroleum ether were made until the percolate was no longer strongly colored. About 20 liters of petroleum ether was used for the complete extraction.

The reddish-brown percolate was concentrated in vacuum on a water bath at a temperature not exceeding 50°C. Much of the petroleum ether was recovered in this process. During the evaporation of the solvent, dry carbon dioxide was passed through the liquid through a finely drawn-out capillary in order to reduce the danger of bumping, to aid in the evaporation of the solvent, and to prevent oxidation of the carotin.

The percolate was concentrated to a volume of approximately 200 cc. and was placed in an ice box at 0°C. for several days. At the end of this time carotin had begun to crystallize out and it was filtered on a hard filter paper under gentle suction to free it from the mother

liquor. The mother liquor was used to rinse the flask in which the precipitation took place in order to remove the crystals of carotin that adhered to the sides of the vessel. The crystals were very small. On rotating the flask they reflected light with a shimmering gold, metallic luster. The color of the mother liquor by transmitted light was deep ruby red but by reflected light was almost black.

The crystals of carotin were dissolved in a very small quantity of carbon bisulfide in the same filter on which they were separated from the mother liquor. The carbon bisulfide solution was caught in a 300-cc. Pyrex distillation flask and successive small quantities of absolute alcohol up to approximately 100 cc. were added with shaking. Reduced pressure was applied and shaking continued by hand until the volume was reduced to approximately 50 cc. This operation required about two hours.

The crystals obtained were filtered and the flask in which the precipitation took place was rinsed with the filtrate and finally with small quantities of petroleum ether. Petroleum ether assisted in removing most of the fats and waxes precipitated with the carotin. Suction was applied to free the crystals from the solvent and an additional small quantity of petroleum ether was employed to wash them again.

The crystals were then dissolved in carbon bisulfide and precipitated by the addition of about 100 cc. of petroleum ether. The purpose of this recrystallization was to eliminate the possibility of the carotin crystals containing alcohol of crystallization. The evaporation of the combined carbon bisulfide and petroleum ether solution of carotin was conducted under vacuum to a volume of 30-40 cc. The crystals were collected on a hard filter paper and washed several times with small portions of low-boiling petroleum ether. The melting point of the crystals, after drying them in a vacuum desiccator for an hour, was 163°C. As this value was much lower than those reported in the literature, the crystals were recrystallized again from carbon bisulfide by petroleum ether. A determination of the melting point yielded a value of 168.5-169°C. (corrected). The latter values for the melting point were the result of two determinations. Palmer (1922) gave the melting point of carotin as 167.5-168°C. Schertz (1925a), Willstätter and Mieg (1907), and Willstätter and Stoll (1928), report a melting point of 174°C. Willstätter and Stoll (1928) stated that the melting point of carotin varied somewhat, depending upon the manner of heating. The carotin crystals sintered a little previous to melting, but this phenomenon alone should not cause the discrepancy between the value obtained and the one most recently reported by Schertz.

The properties of carotin.—Carotin is a highly unsaturated hydro-carbon of the formula $C_{40}H_{56}$. It crystallizes in rhombic prisms from carbon bisulfide on the addition of absolute alcohol, and in quadratic plates from petroleum ether. The color of the crystals is yellow to dark purplish red. Their surface has a metallic luster by reflected light, which was best observed in the precipitated crystals contained in the mother liquor before filtering. Crystals precipitated with absolute alcohol have alcohol of crystallization, which is the reason the final precipitation was carried out with petroleum ether instead.

The solubility of carotin has been determined by Schertz (1925a). He reported that the solubility in absolute alcohol at 25°C. was 15.5 mg. per liter, and 626 mg. per liter in petroleum ether. The instability of carotin in ethyl ether makes solubility determinations rather difficult in this solvent. In ether that has been specially purified, Schertz reported the solubility of carotin at 25°C. as 1005 mg. per liter. Carbon bisulfide and chloroform are the best solvents.

Dilute solutions of carotin in petroleum ether are yellow, but very concentrated solutions are ruby red. Carotin forms a red solution in carbon bisulfide even when dilute.

The stability of carotin in solution was determined by Schertz (1925a). He found that carotin was fairly stable in petroleum ether and alcohol solution as determined by spectrophotometric observations of their transmittancies. In the instance of the two solvents mentioned no appreciable change in concentration occurred after 150 days when the solutions were stored in an ice chest. The solution of carotin in ether, however, was very unstable and decreased in concentration at once very rapidly. The reason for the oxidation of carotin in ether solution may be ascribed to ether peroxide, which it usually contains in small quantity.

Schertz's data on the stability of carotin in petroleum ether solution is especially significant in connection with the study of flour extracts reported in this paper. It can be assumed that gasoline extracts of flour will be stable for a long time if stored with suitable precautions. A gasoline extract of flour stored for 24-48 hours in the dark of a laboratory desk drawer should not change its carotin concentration appreciably. In the data reported on the transmittancy of petroleum ether extracts of flour, the extracts stood for 24 to 48 hours before spectrophotometric observations were made.

Carotin crystals are readily oxidized, whereupon they bleach entirely and their original melting point falls. They are more stable than xanthophyll crystals. Schertz (1925b) showed that the dry sam-

ples of carotin were more readily oxidized in light than in darkness at room temperature. In a comparison of the rates of oxidation of carotin and xanthophyll at room temperatures he showed that in general the carotin in solution as well as in crystalline form was more stable than xanthophyll in the dark and less stable in the light.

The halogen derivatives of carotin will be considered under the discussion of chlorine bleaching. It is sufficient here to state that two iodine derivatives have been prepared of the formulae $C_{40}H_{56}I_3$ and $C_{40}H_{56}I_2$. A bromine derivative having the formula $C_{40}H_{36}Br_{22}$ has also been reported.

The adsorption properties of carotin will be discussed somewhat in connection with the filtration of gasoline extracts of flour. Dissolved in petroleum ether and carbon bisulfide, carotin is not adsorbed by finely divided substances like calcium carbonate, inulin, and powdered sucrose. Charcoal is a good adsorbent, but varies with the character of the charcoal and its source. The fact that xanthophyll is adsorbed from petroleum ether by calcium carbonate serves as a distinguishing characteristic between these two pigments.

The absorption spectrum of carotin is characteristic of this pigment. It has been determined in alcoholic solution by Willstätter and Stoll (1913, 1928). The absorption spectra in petroleum ether, alcohol, and ethyl ether are identical. Xanthophyll has a somewhat similar absorption spectrum but the bands are somewhat displaced toward the violet. Palmer (1922) pointed out that the characteristic feature of the carotin bands is that the solar line F divides the first band almost exactly in half.

The absorption spectra of pure carotin, flour extract, and bran extract.—In order to confirm the observation of Monier-Williams (1912) that carotin was the pigment of wheat flour, it appeared desirable to compare the absorption spectra of a preparation of pure carotin with extracts of flour.

Pure carotin was prepared in the manner previously described. Several gasoline extracts of flours prepared for ordinary transmittancy measurements were combined, and when a large quantity had been secured the solution was concentrated under vacuum. The solution that remained was the one used in the comparisons of the absorption spectra that follow.

Bran extracts were prepared by shaking finely ground bran with high test gasoline and filtering. Before the bran was ground it was carefully washed with water to free it from as much of the adhering endosperm tissue as possible. Washing was continued until a drop

of water squeezed from the wheat bran did not look cloudy on falling into a beaker of clear water. In this manner contamination of the bran extract with the pigment of the endosperm was largely eliminated.

Spectrum photographs of the extracts prepared in the above manner were made through the courtesy of the Physics Department of the University of Minnesota by Mr. E. H. Northey, to whom grateful acknowledgment is made. The instrument used was an Adam Hilger quartz prism spectrograph of the Litlow type, taking a spectrum from 800 to 180 mμ. in 3 ten-inch plates. The light source used for wave length identification was a Pfund iron arc placed 25 cm. from the slit. The light source for absorption work was a 200-watt Mazda lamp placed 25 cm. from the slit. A cylindrical condensing lens of short focus was used in conjunction with both light sources. Photographs were taken on Wratten and Wainwright panchromatic plates. The thickness of the glass absorption cell used for all photographs was 10 cm.

The spectrum photographs are shown in Plates I, II, III, and IV. In Plate I, a comparison of the absorption spectra of petroleum ether solutions of pure carotin is made with the concentrated gasoline extract of flour and the extract of bran prepared as previously described. The absorption spectra are shown between the spectra of the iron arc (Nos. 1 and 6) for wave-length identification. The concentration of pigment in the flour extract was determined spectrophotometrically in the manner reported elsewhere. A solution of pure carotin in petroleum ether was prepared with a similar concentration. The gasoline extract of bran was examined spectrophotometrically and the concentration of its pigment for the purpose was assumed to be entirely carotin. This was an erroneous assumption, as was surmised beforehand, but it was the only way in which comparable pigment concentration of the bran extract could be prepared.

The absorption spectrum of pure carotin in petroleum ether solution is shown in Plate I (No. 2) and Plate II where the persistence of the bands with increasing dilution is also illustrated. Measurements of the positions of these bands show excellent agreement with similar measurements reported by Willstätter and Stoll (1928, p. 222). A comparison of the two is given in Table III.

The absorption spectrum of a concentrated gasoline extract of flour, shown in Plate IV and Plate I, No. 3, has the same characteristic bands as the solution of pure carotin. They are, however, slightly shifted to the left. It is characteristic of xanthophyll bands to be shifted to the left as compared with carotin. Consequently, the absorp-

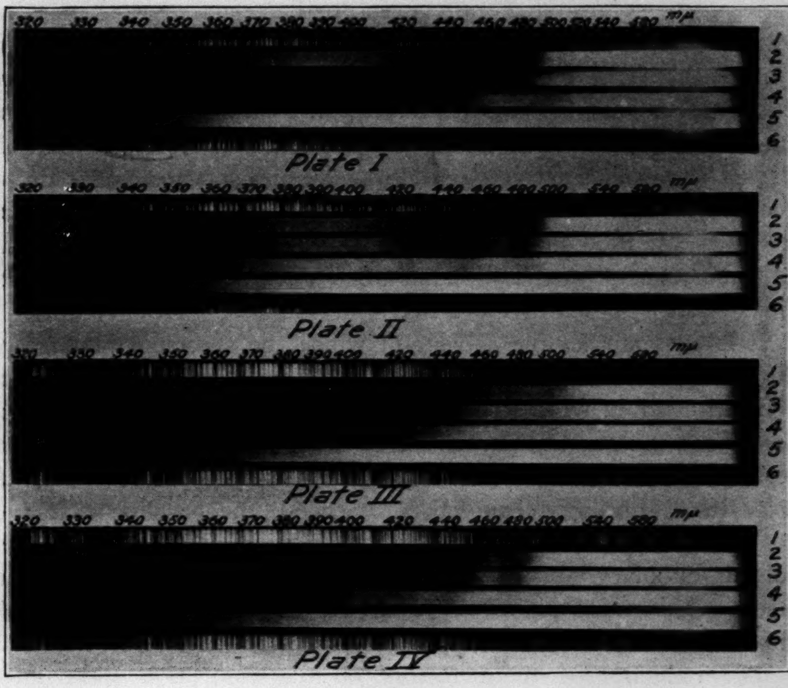

PLATE I

Comparison of the Absorption Spectra of a Petroleum Ether Solution of Pure Carotin, Flour Extract, and Bran Extract. Thickness of Cell in Each Case 10 cm. Exposure 20 Seconds

1. Iron arc spectrum
2. Petroleum ether solution of pure carotin, 0.68 mg. per liter
3. Concentrated gasoline extract of flour; carotin concentration 0.68 mg. per liter
4. Gasoline extract of bran; pigment concentration, 0.68 mg. per liter
5. Solvent (petroleum ether).
6. Iron arc spectrum

PLATE II

Absorption Spectra of Three Dilutions of Pure Carotin in Petroleum Ether Solution. Thickness of Cell in Each Case 10 cm. Exposure 20 seconds

1. Iron arc spectrum
2. Petroleum ether solution of pure carotin, 0.68 mg. per liter
3. Petroleum ether solution of pure carotin, 0.51 mg. per liter
4. Petroleum ether solution of pure carotin, 0.34 mg. per liter
5. Solvent (petroleum ether)
6. Iron arc spectrum

PLATE III

Absorption Spectra of Three Dilutions of the Gasoline Extract of Bran. Thickness of Cell in Each Case 10 cm. Exposure 60 Seconds

1. Iron arc spectrum
2. Gasoline extract of bran; pigment, 0.68 mg. per liter
3. Gasoline extract of bran; pigment, 0.51 mg. per liter
4. Gasoline extract of bran; pigment, 0.34 mg. per liter
5. Solvent (petroleum ether)
6. Iron arc spectrum

PLATE IV

Absorption Spectra of Three Dilutions of the Gasoline Extract of Flour. Thickness of Cell in Each Case 10 cm. Exposure 60 Seconds

1. Iron arc spectrum
2. Gasoline extract of flour; carotin concentration, 0.68 mg. per liter
3. Gasoline extract of flour; carotin concentration, 0.51 mg. per liter
4. Gasoline extract of flour; carotin concentration, 0.34 mg. per liter
5. Solvent (petroleum ether)
6. Iron arc spectrum

tion spectrum secured for the concentrated extract of flour does not constitute absolute proof that the pigment present was carotin alone. However, the shifting of the bands to the left in the case of the gasoline extract of flour, in comparison with pure carotin, may be explained on the basis that the flour extract was concentrated from gasoline solutions. The gasoline extracts of flour were concentrated under greatly reduced pressure at 50°C. until no more material could be volatilized. The density of the concentrate was markedly greater than that of the petroleum ether, as was noticeable when dilutions were made for the various spectra shown in Plate IV.

It is evident that the solvent in which the spectrum photographs of flour pigment were made differed in two important respects from the petroleum ether in which the pure carotin was dissolved for this purpose. First, the gasoline, by virtue of its concentration in the manner described, was reduced to a mixture of heavier hydrocarbons of greater density than pure petroleum ether. Second, the gasoline extract of flour contained, besides the pigment, all the other material that such a solvent dissolves, and as the concentrated extract examined represented a considerable quantity of flour, the amount of flour fat in solution was no doubt a factor.

The influence of these factors on the absorption spectrum of the flour extract compared with the petroleum ether solutions of pure carotin can readily be discerned. It will be noted that the absorption bands in the instance of the flour extract are somewhat more diffused. In addition, general absorption is shown in the spectra of the flour and bran extracts in the region from 420 mμ toward the violet. The absorption spectrum of petroleum ether alone is shown on each of the plates, and the lack of general absorption in this region will be noticed. The same lack of general absorption is likewise apparent in the absorption spectra of pure carotin in which petroleum ether is used as the solvent.

The effect of the solvent is illustrated well in Plate IV, which shows the persistance of the absorption bands in the concentrated gasoline extract of flour with increasing dilution. As increased dilutions with petroleum ether are made, the general absorption due to the heavier, concentrated gasoline extract of flour diminishes. The greater the dilution the less general absorption takes place. Three absorption bands are visible in concentrations of 0.68 and 0.51 mg. per liter of solution. They become scarcely discernible when the dilution of pigment is 0.34 mg. per liter. Band III shows plainly at a concentration of 0.51 mg. per liter.

TABLE III

COMPARISON OF THE ABSORPTION SPECTRUM OF A PETROLEUM ETHER SOLUTION OF PURE CAROTIN, WITH THE ABSORPTION SPECTRA OF GASOLINE EXTRACTS OF FLOUR AND BRAN THICKNESS OF CELL 10 CM. CONCENTRATION OF ALL SAMPLES 0.68 MG. PER LITER

	Pure carotin	Flour extract	Bran extract
Band I	493-477	488-467	502
Band II	460-442	455-443	(Very slight absorption)
Band III	429-414	425-411	

WILLSTATTER AND STOLL'S DETERMINATION OF THE ABSORPTION SPECTRUM OF PURE CAROTIN IN ALCOHOLIC SOLUTION USING A GRATING SPECTROSCOPE
CONCENTRATION OF CAROTIN 5 MG. PER LITER

	5 - cm. Cell	10 - cm. Cell
Band I	492 - 478	492 - 476
Band II	459 - 446	459 - 445
Band III	415	419

The gasoline extract of washed bran gave a spectrum with no similarity to the absorption spectrum of either carotin or flour extract. It is therefore evident that the bran pigment is not carotin. The gasoline extract was, however, highly pigmented. Its hue was very different from the hue of the carotin solutions, the bran extract having a decidedly reddish color. It is not exactly clear what constitutes the pigment of bran. Solutions of xanthophyll are reported in the literature as yellow and this, coupled with the fact that xanthophyll is almost insoluble in petroleum ether, makes it appear unlikely that it is the pigment of bran.

The measurements of the width of the absorption bands for pure carotin, flour extract, and bran extract are given in Table III.

These comparisons are important as the validity of the method described for the quantitative measure of the yellow pigments of gasoline extracts of flour depends largely upon whether or not carotin is the sole or principal pigment of such an extract. The photographs of the absorption spectra indicate that this is the case. At any rate the flour extract exhibits the characteristics of a carotin solution to an extent that allows the assumption that the principal pigment is carotin. When an attempt was made to fractionate the flour pigment using the differential adsorption method of Tswett, known as the chromatographic analysis, using fine dry calcium carbonate as the adsorbent (Palmer 1922, page 226), no yellow ring, characteristic of xanthophyll, was produced. This tends to confirm the assumption that little, if any, xanthophyll was present in mixture with the carotin in our gasoline extracts.

Even if xanthophyll were present to an appreciable extent in some instances, the curve showing the relation between the per cent transmittancy and carotin concentration would not vary greatly from the

curve for pure carotin (See Fig. I) over the range in which flour extracts are examined. For example, if an ordinary unbleached cample of straight grade flour with a transmittancy of 12 per cent contained as much as one-third xanthophyll, the error in the assumption that the coloring matter was entirely carotin would amount to only 0.067 parts in one million.

Should it be shown by later investigation that xanthophyll is contained in flour to an appreciable extent, it may be advisable to calculate a correction for the carotin curve based on these findings. The principle involved in the determination would, however, require no alteration, but a correction of small magnitude only would need to be applied.

Plate II shows the effect of dilution on the persistence of the absorption bands of pure carotin in petroleum ether solution. The bands are extremely faint at a dilution of 0.24 mg. per liter.

The influence of dilution on the absorption spectra of bran extract is shown in Plate III. At none of the dilutions used could any characteristic bands be discerned. A faint suggestion of a band appears in the neighborhood of 500 mμ.

A preliminary study has been made of the spectral distribution curves for petroleum ether extracts of flour and washed bran in comparison with solutions of pure carotin in the same solvent. It was found that the bran pigment had spectral distribution properties distinctly different from those of carotin. Carotin, if present at all, constituted such a small proportion of the soluble pigments as to be completely masked. This difference between carotin and the bran extract was pointed out under the discussion of the spectrographs of these substances. The spectral distribution curve for the flour extract possessed carotin characteristics, but evidence was obtained of the presence of another pigment in addition to carotin. The flour extract curve was, however, sufficiently characteristic of carotin to justify the supposition that the pigment of flour was essentially carotinoid. More complete data on this point are being obtained.

Summary

It has been shown that pure carotin concentration can be measured by determining the transmittancy at wave length 435.8 mμ of its petroleum ether solution. Further, a reasonable basis for the assumption that dilute solutions of carotin and petroleum ether extracts of flour are similar has been established. These facts permit the correlation of the transmittancy of flour extracts with carotin concentration in accordance with the procedure described for pure carotin. It has been

shown that the presence of a small quantity of another pigment like xanthophyll would not cause an error of any significance in the estimation of flour color expressed as carotin alone. For the purpose of flour color measurement the assumption is made, therefore, that the flour pigment is carotin. In carrying out such measurements the transmittancy of a gasoline extract of flour is determined. The concentration of pigment expressed in milligrams of carotin per liter of solution is learned by consulting the curve (Fig. 1) showing the relation between per cent transmittancy and carotin concentration. The concentration of pigment in the flour is then computed from the proportion of flour and solvent used in preparing the extract, and the results are expressed as parts of carotin per million parts of flour. The detailed procedure will follow.

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ERRATA

In the right hand column head of Tables VI and VII on pages 146 and 147 of the March, 1929, issue, the greek letter eta, η, as it appears as the first factor in the numerator should be replaced by the greek letter pi, π. A similar correction should be made in the shearing stress formula below Table VI on page 146, and in the last line on page 146.

A QUICK VISCOSIMETRIC METHOD FOR MEASURING THE STALENESS OF BREAD

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(Received for publication April 1, 1929)

The final goal of the investigations dealing with the staling of bread would be, without doubt, to find a procedure that would delay or even wholly prevent this process. To accomplish this it is not enough to know the cause or causes that produce the phenomenon, but a method must be developed by means of which, for instance, the effect of the addition of certain suitable substances would be easily observable.

Altho experiments have been made toward this end, we do not as yet know a method that would fulfill the required conditions in every respect. The decrease during staling of the quantity of carbohydrates soluble in water but insoluble in alcohol is unsuitable for this purpose, the determination being too complicated and lengthy. But the swelling capacity seems to be very useful. Three methods are commonly used for the determination of this: (1) The weight, (2) the volumetric or sedimentary, and (3) the viscosimetric method. The first of these three is not applicable to bread for obvious reasons. The use of the second method was elaborated in detail by J. R. Katz (1915). Altho we get easily measured differences between fresh and stale bread with this method, its chief drawback is that the determination requires a whole day and, compared with an eventual viscosimetric method, one must concede the preference to the latter. To elaborate such a method seemed therefore to be a most suitable and also a very promising task.

The writer's experiments were based on the researches of Lüers and Ostwald (1919), which deal with the viscosity of solutions of dough and of flour paste. They adapted to their measurements Wilhelm Ostwald's viscosimeter, but in a somewhat modified form. The exact dimensions of this modified viscosimeter were not indicated clearly in Lüers' and Ostwald's publications so the writer ordered a specimen viscosimeter from the firm mentioned by them (Messrs. R. Götze, in Leipzig) that made their viscosimeters. Varying gradually the capillary of the instrument, but leaving all other dimensions the same, the writer had a set of viscosimeters manufactured for his orienting experiments. The aim of the experiments was to determine what factors must be taken

into consideration in measuring the water suspension of wheat bread; what concentration is most suitable, and in what water-equivalent viscosimeter.

One of the most important demands is the careful maintenance of the temperature. It is very interesting that the unpleasant circumstance occuring in the experiments with starch solutions and solutions of dough from wheaten flour, i.e., the constant decrease of viscosity with time, is absolutely absent from the 10% suspension of the wheat bread. The viscosity of the ready suspension remained practically unaltered, if evaporation is checked by addition of a few drops of toluene. Comparisons of concentration were made with suspensions of 5, 10, 15, and 20%. The 5% suspension was found to be not sufficiently sensible; definitely measureable differences were found at a concentration of 10%, and those at 15% and 20% were still more so. The preparation of the last two suspensions is somewhat troublesome, however, the disposable quantity of water, especially in the case of the 20% suspension, being hardly sufficient for careful work. The writer has found the 10% suspension best suited to these purposes. It is easily prepared and the sensibility is wholly sufficient.

Considering, finally, the speed of flow, the writer has found that best results were obtained if this is 150-300 sec/5. If the speed is much below 150, accuracy of timing is difficult; if much above 300, the speed of the inevitable sedimenting of the coarser particles can easily go beyond the speed of flow. Using the 10% suspension, the viscosimeter most suitable had a water-equivalent of 70-80 sec/5. The viscosimeter used in the experiments given below had a w.e. of 74 sec/5.

With these facts in mind the writer has adopted the following method: 10 grams of the crumb of bread was broken into smaller pieces and wetted with distilled water; the pieces were pressed first with a flat, smooth, glass stopper, later with a brush through a Dufour's "No. 5, XXX, triple extra" silk sieve about 10 cm. in diameter into a flat porcelain dish of known weight. After carefully washing the used instruments and squeezing the lumps, the suspension was completed with water to 100 grams (10 grams of crumb + the used water, weighed to an accuracy of 2 decimals) after stirring in a 250 ccm. Erlenmeyer flask the viscosimeter was filled with it up to the graduation. After corking the tubes, the viscometer was placed in a large water bath, which was kept at a temperature of 20°C. Having acquired the desired temperature, the suspension was made uniform by blowing a few air

bubbles through it, and then measuring in sec/5 the time of flow. An average value was taken of at least four readings.

The reproducibility is very good. Only in extreme cases was a difference of more than 2 sec/5 observed between replicated observations.

Out of the many experiments, the writer offers as examples the following results of his measurements. Two equal loaves for these experiments were made from the different flours with water, salt, and yeast. After cooling, these were weighed, a 10-gram average sample was removed from the crumb of one, and the other was put aside untouched. The measurements with the material were made by the method given above. The other loaf, after being stored for 48 hours, was treated in the same manner, but the loss of weight was taken into consideration. The results are given in Table I.

TABLE I

VISCOSITY OF 10% SUSPENSIONS OF FRESH AND STALE WHEAT BREADS

Expt.	Flour No.	Fresh		After 48 hours		
		sec/5	T/t*	sec/5	T/t*	Decrease %
1	Os	270	3.649	171	2.311	36.67
2	Os	258	3.486	166	2.243	35.67
3	Og	254	3.432	164	2.216	35.43
4	Og	267	3.608	168	2.270	37.08
5	4	237	3.203	157	2.122	33.69
6	4	265	3.581	173	2.338	34.72
7	4	273	3.689	179	2.419	34.43
8	4	278	3.757	189	2.554	32.01

*T is the measured relative viscosity of the suspension, t the water equivalent of the viscosimeter, in this case 74 sec/5.

In the last column the decrease of the viscosity of the bread stored for 48 hours is given in percentages of the viscosity of the fresh bread. The writer has not elaborated on the question of the progress of the staling during the intervening time, this being satisfactorily known, especially through the works of Katz.

The given method, as can be seen, is simple, sensible, and quick, because, compared with the sedimentary method of Katz, which requires 24 hours, the measurement, including the time required to bring the temperature of the suspension to 20°C., can easily be finished within 40 minutes.

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A GLUTEN WASHING MACHINE

By T. R. James

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(Received for publication March 25, 1929)

During the past several years there has been evolved in this laboratory a fairly satisfactory machine for the analytical washing of flour glutens. Figure 1 shows a top view of the machine and Figure 2 is a cross-section along the line CS.

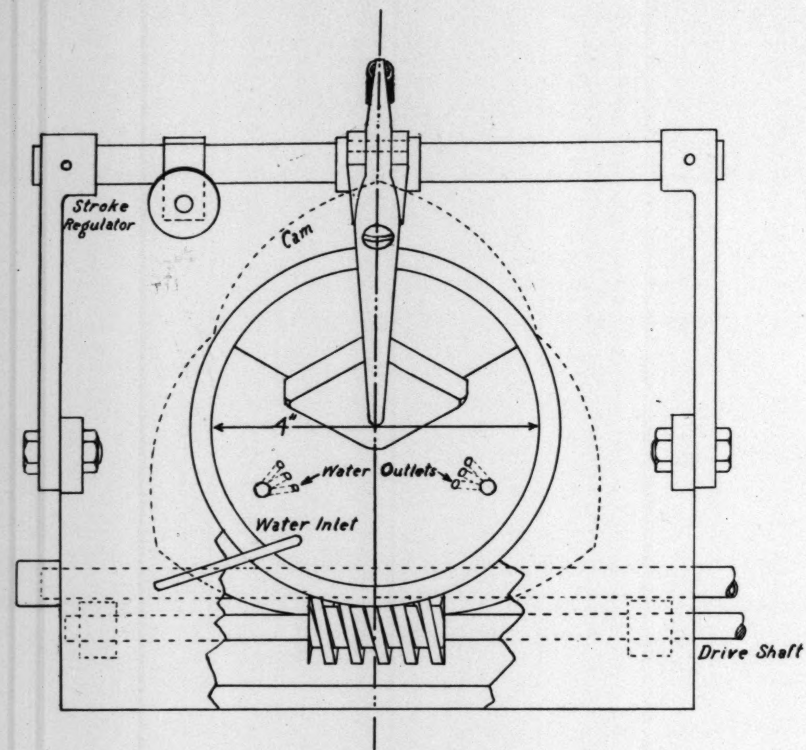

Fig. 1. Top View of Gluten Washer

The gluten is washed in a revolving cup by a reciprocating paddle, the paddle making three strokes to each revolution of the cup. The inside of the cup is shaped like the corner of a cube intersected by a perpendicular cylinder. The line abc in Figure 2 is where the plane surface formed by one side of the cube corner intersects the cylinder. With this kind of cup the dough ball is squeezed in turn against three

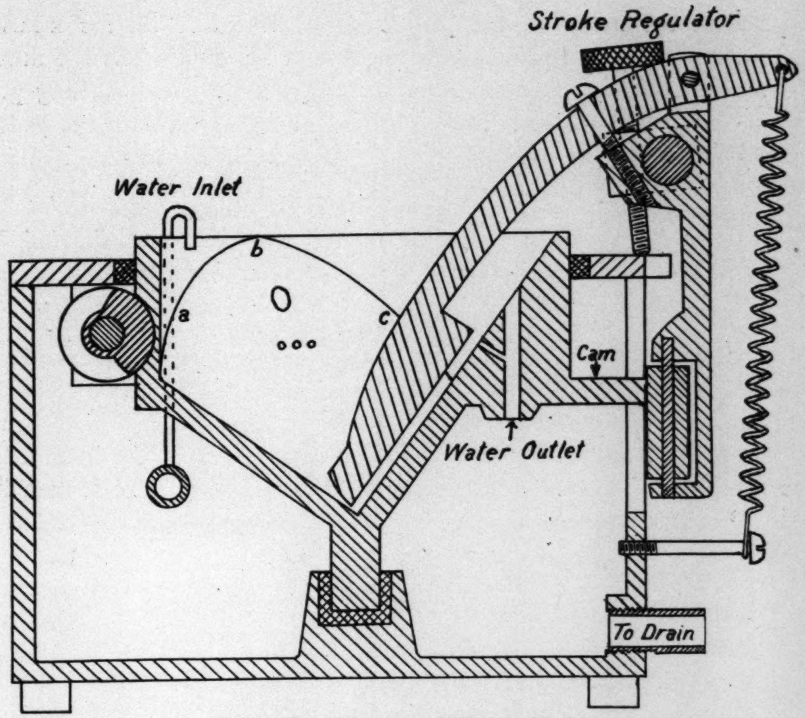

Fig. 2. Cross Section of Gluten Washer

plane surfaces at right angles with each other. This method of washing tends to keep the ball together and evenly kneads all parts of it.

The cup is revolved by a worm, operating against gear teeth on the outer circumference of the cup. The drive shaft may be directly connected to a motor or belt driven. The cam which surrounds the cup operates the paddle. The stroke of the paddle can be adjusted by the knurled thumb nut while the machine is operating. It is desirable to have two operating speeds, one about 20 revolutions per minute of the cup and the other about 40 revolutions per minute. However, if time is no object, one speed of about 25 revolutions per minute will be found satisfactory.

The cup and paddles were cast of aluminum. The worm for driving the cup was made of brass. Gear teeth were cut into the outer surface of the cup. Altho aluminum is ordinarily not to be recommended for gears, in this case the teeth are so large in proportion to the force against them that the aluminum gear has been found to wear well. The cam for operating the paddle is a part of the cup casting.

The machine shown is for a 10-gram sample of flour. The dough is made up and allowed to stand under water as usual. When ready

to place in the machine, the paddle stroke is raised by the knurled thumb nut so that the paddle does not flatten the dough ball too much. The machine is then started at the lower speed. As the washing progresses the paddle stroke is lowered two or three times until the paddle comes down hard on the gluten ball. When the gluten ball is fairly well formed, the speed of the machine is increased to about 40 revolutions of the cup per minute and the gluten ball thoroly washed for 10 or 15 minutes to completely remove the starch.

The machine will successfully wash a gluten from most any type of sound flour, altho very weak glutens usually have to be done entirely at low speed. It has been found best to turn on the water so that it barely drops at first. Then as soon as the gluten ball begins to form, the water can be turned on fairly strong. Washing is not as fast as by hand, but several washing cups can easily be included in one machine. Those we made contained three cups in each machine. Also the machine requires very little operator's time, so there is considerable saving in using it from this standpoint.

PHOTOGRAPHS FOR PUBLICATION

By C. G. Harrel

Bakeries Service Corporation

Jamaica, N. Y.

(Received for publication January 30, 1929)

The initial production and the final reproduction of photographs for Cereal Chemistry is somewhat of an expensive process. Whatever the expense amounts to, it is desirable to have the photographs convey to the readers as much information as possible. It is often desirable to know the extent to which the object photographed has been enlarged or diminished. This could be accomplished by stating numerically the degree of enlargement or reduction. This requires certain measurements when the negative is produced and probably some calculation when the reproduction is made.

In practically every reproduction appearing in Cereal Chemistry, there are certain waste spaces around the edges of the picture. These waste edges can by the insertion of a ruler be made to give the photograph a greatly increased value. Reference is made to the paper entitled "Factors Influencing Checking in Biscuits" by J. A. Dunn and C. H. Bailey.[1] When a linear measure is thus introduced in the

[1] Cereal Chemistry Vol. V, No. 5, Page 396.

original photograph, two distinct advantages are gained. First, from any print, the degree to which the photographed object has been enlarged or diminished can be quickly determined. Second, by the use of parallel lines or a pair of dividers many dimensions can quickly be determined by reference to the photographed rule.

A rule made from dull finished white paper having the scale drawn with India ink photographs well. The metric system should preferably be used. When photographing bread, by placing the ruler vertically, the loaf height can easily be read. Care must be taken that the ruler is the same distance from the camera lens as is the point or points of interest in the photographed object.

Photographic prints should be on glossy finish paper and both print and negative should be developed in as "contrasty" a manner as possible since some detail is lost in converting the illustration into a half-tone.

It is urged that cereal chemists give this suggestion consideration as it is believed that by its use their photographs will have an increased value.

Editor's Note.

Supplementing Mr. Harrel's timely comments on photographs, the editors desire to add certain suggestions respecting the preparation of charts or graphic records.

Cross-section paper printed with light blue ink should be used in the preparation of all graphs intended for publication. All lines that are to reproduce must be drawn with black ink. Lettering on the charts should be limited to the numerical expression of the coordinate values. If the services of a competent draftsman are not available, indicate these values with a soft pencil and the necessary lettering will be done in the office of the editor. Do not letter the legends or description of the data on the charts. These legends will be set in type and printed below the charts. Authors must provide a legend for each chart, however, typed on an accompanying sheet, which legend describes the chart in sufficient detail so that reference need not be made to the text for a complete understanding of the source and significance of the data thus expressed